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TITLE: A New Paradigm for the Treatment of Ovarian Cancer: The Use of Epigenetic Therapy to Sensitize Patients to Immunotherapy and Chemotherapy

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13. SUPPLEMENTARY NOTES

14. ABSTRACT: The overall goal of this project remains to bring epigenetic therapy strategies to have major impact for the management of advanced ovarian cancer (OC). In the first year, we have made exciting advances for this purpose and especially for the concept of utilizing low dose epigenetic therapy to target DNA demethylation and histone deacetylase inhibition (HDACi) to maximize responses to immune checkpoint therapy. We identified, from studies of multiple OC cell lines how low doses of the DNA demethylation agents, azacytidine (AZA) and deoxy-azacytidine (DAC) induce a deep immune attraction signature. We completed, in 2014, compilation of a 300 gene panel, we termed AIM (AZA Induced Immune Genes). Next, in 2015, we identified a highly functional panel core wherein AZA induces an interferon triggering pathway involving upregulation of a cytosolic double stranded RNA(dsRNA) sensing system involving up-regulation of endogenous retroviral transcripts (ERV's). These discoveries provide key insight into how epigenetic therapy may help reverse immune evasion to help sensitize to immune checkpoint therapy for OC, and a biomarker system for potentially predicting patient responses and monitoring therapy.

15. SUBJECT TERMS – key words or phrases identifying major concepts Epigenetic therapy, advanced ovarian cancer, DNA demethylation, histone deactylases, immune checkpoint therapy, viral defense, immune cell attraction

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1. INTRODUCTION

The goal of our project remains to develop eventual "epigenetic" therapy strategies, with relatively low toxicities, which can potentially robustly extend the life expectancy of women with advanced ovarian cancer (OC). Underlying this concept is the fact that it is easier to reverse epigenetic abnormalities than to directly correct genetic changes comprised of mutations. Certainly the pathways which the mutations trigger can be targeted by a growing number of drugs. For some, initial responses are robust but for most, relatively rapid emergence of resistance is a problem. Epigenetic therapy can potentially restore gene function which may help normalize multiple pathways for which abnormalities drive cancer initiation and/or progression. These can include pathway abnormalities mediating drug resistance. In this proposal, we are following an emerging potential we have produced in advanced lung cancer, both in the laboratory and clinical trials, to bring our concepts fully to bear on the treatment of OC. This past year, we have concentrated particularly on **Specific Aim 3:** *to study how epigenetic therapy may sensitize OC cells to subsequent immunotherapies which targets checkpoints which are driving immune tolerance*. We have, with participants of key leaders, Cindy Zahnow, Dennis Slamon, and Drew Pardoll, with much lead work from the mentored proposal for this project, Kate Chiappinelli, made exciting progress for **Major Task 1**: *to develop the in-vitro pre-clinical systems to outline the sensitivities and derive molecular signatures that track with these,* and **Major Task 2:** *to develop in-vivo pre-clinical systems to outline the potential efficacy of epigenetic therapy sensitization to immunotherapy for targeting checkpoints which drive immune tolerance*. The major findings, which will be detailed in progress report sections below, involve discoveries providing key insight into how epigenetic therapy may help reverse immune evasion to help sensitize to immune checkpoint therapy for OC, and a biomarker system for potentially predicting patient responses and monitoring therapy. Other key progress aspects will also be outlined.

2. KEYWORDS

1) epigenetic therapy; 2) DNA demethylation; 3) histone deacetylases; 4) immune evasion; 5) immune checkpoint therapy; 6) immune attraction.

3. ACCOMPLISHMENTS

What were the major goals and objectives of the project?

The overall goals remain identical to those outlined in the original proposal. This past year, as introduced earlier, we have concentrated particularly on:

A. Specific Aim 3: *to study how epigenetic therapy may sensitize OC cells to subsequent immunotherapies which targets checkpoints which are driving immune tolerance*. We have, with participants of key leaders, Cindy Zahnow, Dennis Slamon, and Drew Pardoll, with much lead work from the mentored postdoctoral fellow for our project, Kate Chiappinelli, made exciting progress for **Major Task 1**: *to develop the in-vitro pre-clinical systems to outline the sensitivities and derive molecular signatures that track with these,* and **Major Task 2:** *to develop in-vivo pre-clinical systems to outline the potential efficacy of epigenetic therapy sensitization to immunotherapy for targeting checkpoints which drive immune tolerance*. The major findings, as detailed directly below, involve discoveries providing key insight into how epigenetic therapy may help reverse immune evasion to help sensitize to immune checkpoint therapy for OC, and a biomarker system for potentially predicting patient responses and monitoring therapy.

***What was accomplished under these goals*?**

A. Specific Aim 3: Major Task 1: *to develop the in-vitro pre-clinical systems to outline the sensitivities and derive molecular signatures that track with these.* Progress is as follows:

1. *Completion of preliminary work begun in 2013, and 2014 leading to compilation of a 300 gene panel, we termed AIM (AZA Induced Immune Genes)*. In these studies, our mentored fellow, Dr. Chiappinelli is co-first author (OncoTarget, 2014). The enrichment for these genes is highest in OC lines among all cancer types tested including also non-small cell lung cancer, breast, and colon cancer. The gene panel identifies high and low expression groups in primary tumors in OC, TCGA samples and this has proven key to our continued studies outlined in section **2** just below.

2. *Identifying a cytosolic double stranded RNA (dsRNA) viral defense pathway as a core functional circuit for AZA induced interferon response in OC cells and its potential for predicting responses to immune checkpoint therapy.* This has been a major accomplishment during the first year of this award and the work has just been published in Cell, Aug. 2015, with our mentored fellow, Kate Chiappinelli as the first author. A summary of the findings (**Fig.1**), is as follows.

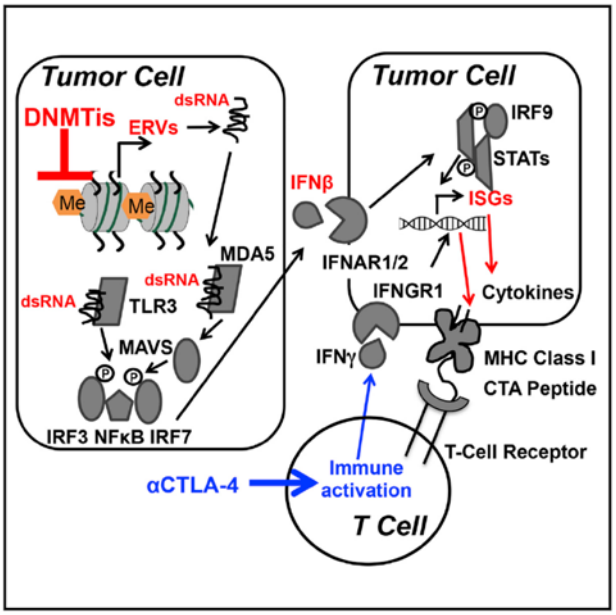


Fig. 1. DNA methyltransferase inhibitors (DNMTis) inhibit DNA methylation of LTR's for endogenous retroviruses (ERVs) to upregulate their transcription in OC tumor cells to induce a growth-inhibiting immune response. High expression of the genes associated with the anti-viral response have the potential to sensitize to immune checkpoint therapy which blocks ligand mediation of immune tolerance including CTLA-4 and PD-L1.

We show that DNA methyltransferase inhibitors (DNMTis) upregulate immune signaling in cancer through a viral defense pathway. In ovarian cancer (OC), DNMTis trigger this pathway via cytosolic sensing of double stranded RNA (dsRNA) causing a type I interferon response and apoptosis. Knocking down sRNA sensors TLR3 and MAVS reduces this response 2-fold and blocking interferon beta or its receptor, IFNAR ½, abrogates it. Upregulation of hypermethylated endogenous retrovirus (ERV) genes accompanies the response and ERV overexpression activates the response. Basal levels of ERV and viral defense gene expression significantly correlate in primary OC and the latter signature separates primary samples for multiple tumor types from The Cancer Genome Atlas into low versus high expression groups (**Fig. 2**). In melanoma patients treated with an immune checkpoint therapy, high viral defense signature expression in tumors significantly associates with durable clinical response and DNMTi treatment sensitizes to anti-CTLA4 therapy in a preclinical melanoma model (**Fig. 3**).

B. Specific Aim 3: Major Task 2: *to develop in-vivo pre-clinical systems to outline the potential efficacy of epigenetic therapy sensitization to immunotherapy for targeting checkpoints which drive immune tolerance*. We are, with Cindy Zahnow leading the efforts and with collaboration from Drs. Drapkin and Pardoll making great progress in this task being well ahead for goals aimed for completion by the end of year 2. Specific accomplishments are:

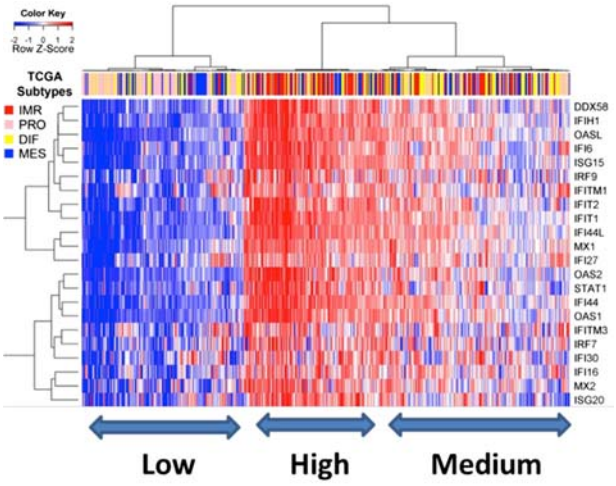


Figure 2. Heatmap comparing basal RNA-seq levels of viral defense genes and ERVs in primary TCGA OC samples. Viral defense gene expression (right Y- axis). For clusters (k = 6), differences are significant between the high ERV expression (2.5 ± 0.37) and the low ERV expression cohort (5.33 ± 0.28). Red bars at top are TCGA tumors classified as immunogenic and cluster with a higher basal viral defense gene expression signature. Pink bars at the left top are tumors classified as proliferative, poor prognosis, and poorly immunogenic OC and have the lowest expression signature.

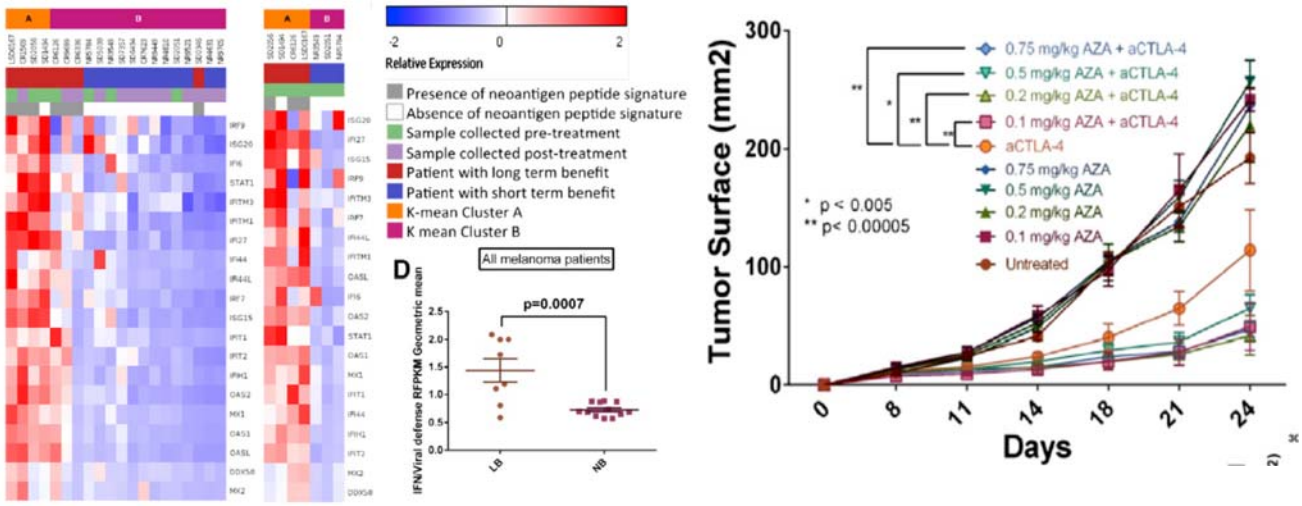


Fig.3. (left panel): Higher expression of Aza-Upregulated viral defense genes (red) correlates, in tumor RNA-seq data from anti-CTLA-4-treated patients having advanced melanoma, with durable clinical benefit (complete response, partial response, or progression free-survival >6 months). Tumors collected pre-CTLA-4 treatment and shortly post-treatment are shown. y axis = RFPKM mean of viral defense genes in all melanoma patients. (Right panel) Tumor responses of mice injected with B16-F10 cells and treated with either PBS, anti-CTLA-4, Aza, or both anti-CTLA-4 and Aza. Data represent results from one of two independent experiments with identical results, each with n = 10 per arm. Y axis = mean tumor surface, error bars ± SEM.

1. *Implementing a syngeneic mouse model to study, in-vitro and in-vivo the potential efficacy of epigenetic therapy sensitization to immunotherapy for targeting checkpoints which drive immune tolerance*. Under the leadership of Dr. Zahnow, and with, again, much participation from our mentored post-doctoral fellow, Dr. Chiappinelli and a graduate student, Meredith Stone, we have successfully implemented the MOSE mouse model of serous OC and made extensive progress at a pace must faster than we anticipated. In this model, the mice receive tumor cells intraperitoneally and develop ascites in a manner similar to what can occur in patients with advanced OC. This model is known to be poorly immunogenic giving us the opportunity to determine whether our epigenetic therapy strategies can alter this scenario and sensitize to immune checkpoint therapy in so doing. The following important data have emerged:

a. AZA treatment of MOSE cells leads to up-regulation of the viral defense genes seen in human OC and other cancers (**Fig. 4**): These results suggest good conservation between human and mouse tumor cells for the pathway induced by AZA which we propose has a major role in our scenario for sensitizing to immune checkpoint therapy.

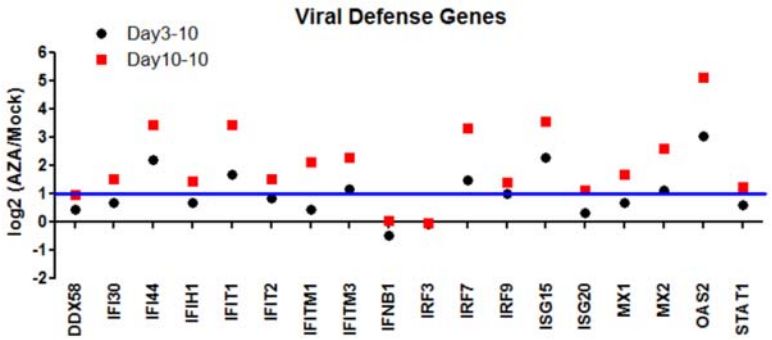


Fig. 4. MOSE ID8 cells were grown in vitro and treated with 500 nM AZA for 10 days (orange squares), or for 3 days (black circles) with 7 days of rest. At days 3 and 10 cell pellets were collected and sent for array expression analyses. Results are shown for the post-treatment changes over mock (Y-axis) for the viral defense genes shown on the X-axis.

b. Pre-treatment of MOSE OC cells leads to attraction of immune cells in-vitro (**Fig. 5**): The same AZA vs mock treatment protocol shown in Fig. 4 has been used to treat the MOSE cells placed in the bottom of a transwell chamber. Untreated mouse immune cells are then placed in the top chamber and then exposed for 4 hours to the treated cells in the lower chamber. An AZA induced time dependent migration of the immune cells to the bottom well is seen (**Fig. 5**). These data indicate that AZA triggers a tumor communication which attracts host immune cells and this may involve the drug induced viral defense, interferon signaling outlined in the sections above.

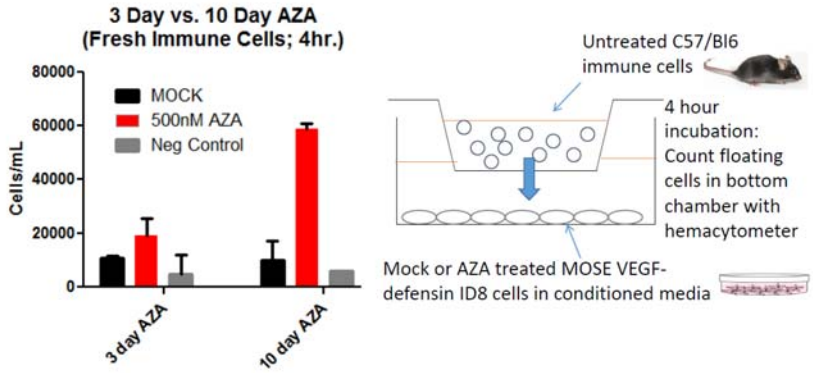


Fig. 5. Effects of AZA treatment on ability of MOSE OC cells to attract immune cells. Note AZA induced increases in migration in red.

c. MOSE OC cells pre-treated with AZA and placed into mice increases the recruitment of immune cells into ascites fluid (**Fig. 6**). The immune attraction results seen in the above transwell chamber studies can be extrapolated to an in-vivo setting. When the above in-vitro exposure protocol is used to treat MOSE cells with 500 nM AZA for 10 days, and then the cells are injected into the abdomen of mice, ascites develops less rapidly in the pre-treated cells (**Fig. 6 – top left panel)**. Furthermore, the concentration of immune CD3 cells is markedly increased in the ascites from mice injected with the pre-treated vs mock treated cells (**Fig. 6 – bottom left panel)**. Ex-vivo treatment of the cells with the histone deactylase inhibitor (HDACi) entinostat, at several doses for 3 and 10 days, does not cause either of the above affects (**Fig. 6 – right upper and bottom panels)**.

d. Developing the above mouse model for in-vivo treatment employing epigenetic therapy to sensitize to immune checkpoint therapy. In this most important aspect of our mouse work, we have, again, made great progress and we are way ahead of the predicted development of this from year 2 to 4. We are testing not only an initial regimen with low dose, 0.5 mg/kg, AZA alone, but also two different HDACi, each alone and in combination with AZA. These latter two include entinostat which is being used in our ongoing trials with NSCLC, and givinostat. While the former has an excellent Ki against HDAC1, the latter is even better and also targets HDAC2. These two HDAC's, from our recent work are the two for which inhibition is most involved in working additively with AZA to achieve re-expression of abnormally silenced, or low expression, cancer genes. In a first study with NSCLC cells, the combination of givinostat and AZA is the best for up-regulating the viral defense genes and also selected ERV's. Using this information, we have developed treatment regimens wherein the HDACi's are administered chronically after AZA at low doses. This is different than has been done in the clinic with HDACis previously where higher doses are been given only intermittently.

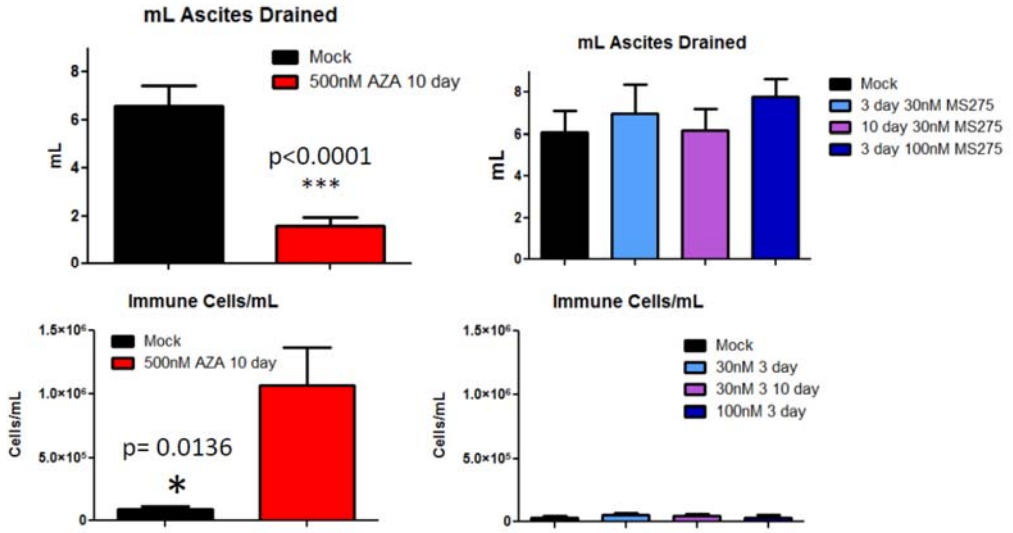


Fig. 6. Effects of 500 nM AZA or 30 and 100 nM entinostat pre-treatment on ability of MOSE OC cells to attract immune cells into ascites when explanted in-vivo.

Our hypothesis is that our combined regimen employing the low dose chronic HDACis will be tolerable and provide a more chronic pressure on chromatin to enhance the viral defense gene and ERV up-regulation which may be key for sensitization to immune checkpoint therapy. Our results to date are as follows:

1) *Treatment model (**Fig. 7**)*. The schema used for all sections below is shown.

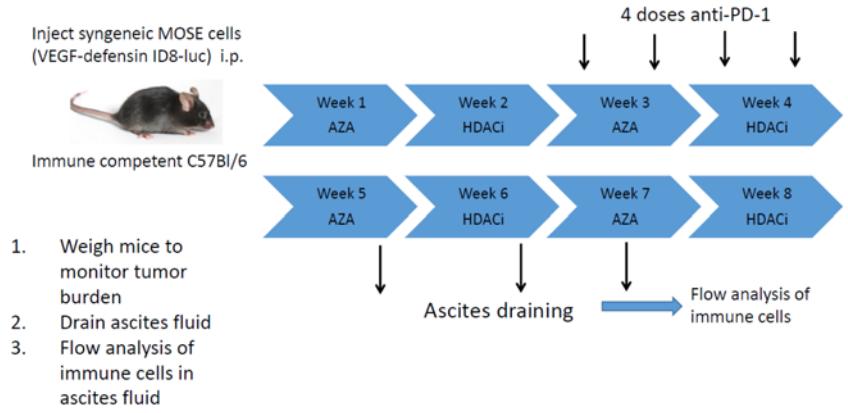


Fig. 7. Effects of 500 nM AZA or 30 and 100 nM entinostat pre-treatment on ability of MOSE OC cells to attract immune cells into ascites when explanted in-vivo.

2) *Ascites is best reduced with AZA combined with anti-PD-1 and HDAC inhibitors (**Fig. 8**)*.

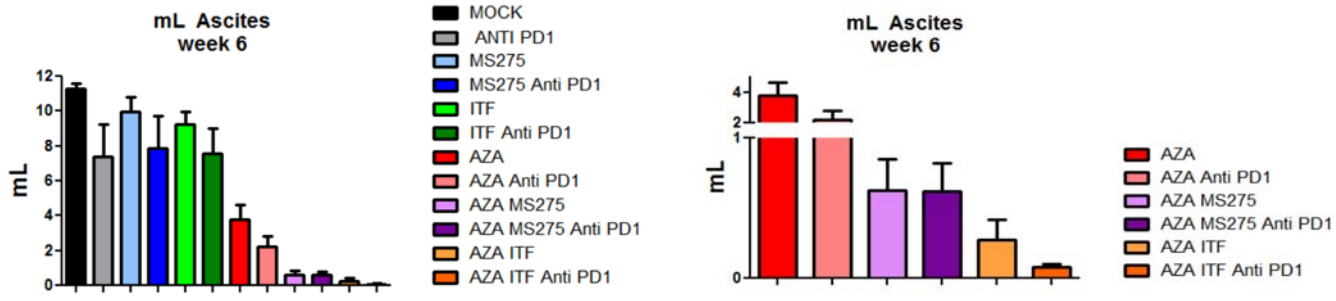


Fig. 8. Effects of in-vivo treatment with the schema in Fig. 7 of AZA and/or entinostat (MS275) and/or givinostat (ITF) on the accumulation of ascites. Left panel – Ascites measurements with various drug administrations alone or in combination (0.5mg/kg for AZA, and 2mg for the HDACi's). Right panel – View expanded for all results with AZA alone or in combination as shown. Note most ascites decrease with the AZA-ITF-anti-PD1 combination.

3) *In vivo AZA combined with MS275 or ITF, and/or anti-PD-1 significantly increases survival in mice (**Fig. 9**).*

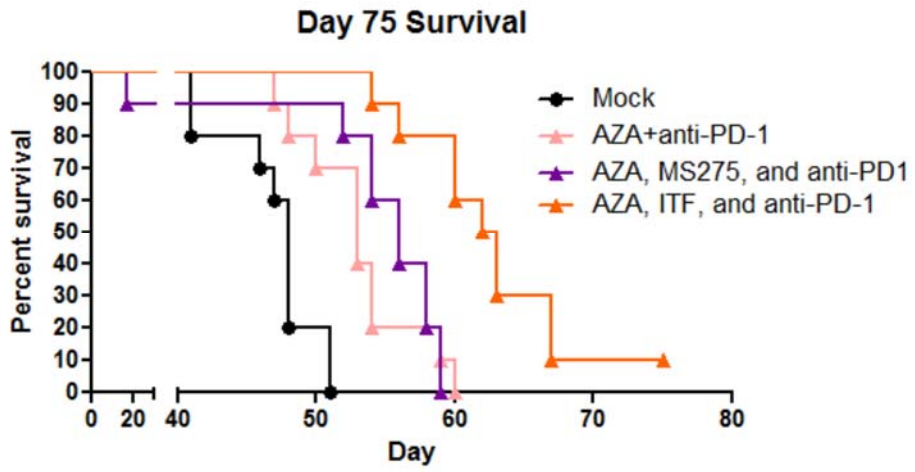


Fig. 9. Effects of in-vivo treatment with the schema in Fig. 7 of AZA and/or entinostat (MS275) and givinostat (ITF) on survival of the mice. Sacrifice is always based on mouse discomfort from ascites accumulation.

4) *In vivo AZA treatment combined with anti-PD-1 and/or HDAC inhibitors increases CD3+ T cells in the tumor environment, and increases activated (IFNγ+) CD4 and CD8 T cells. The increased activation of T cells is specific to AZA and/or plus HDACi and anti-PD-1 (**Fig. 10**).*

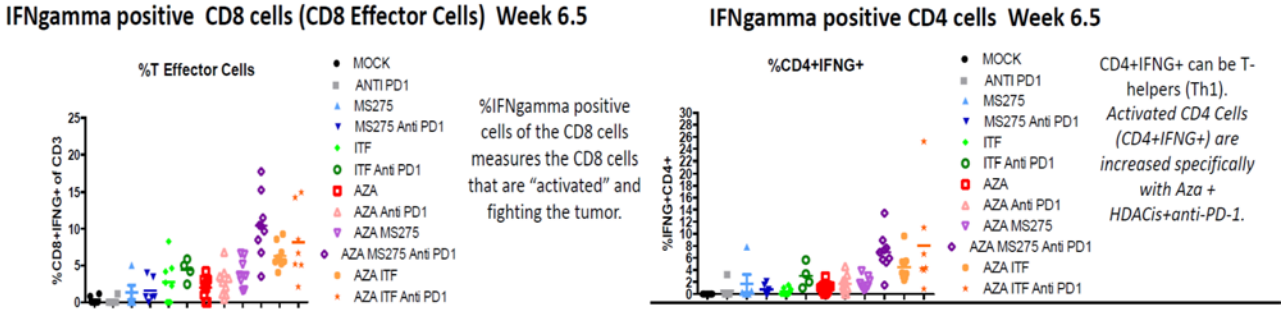


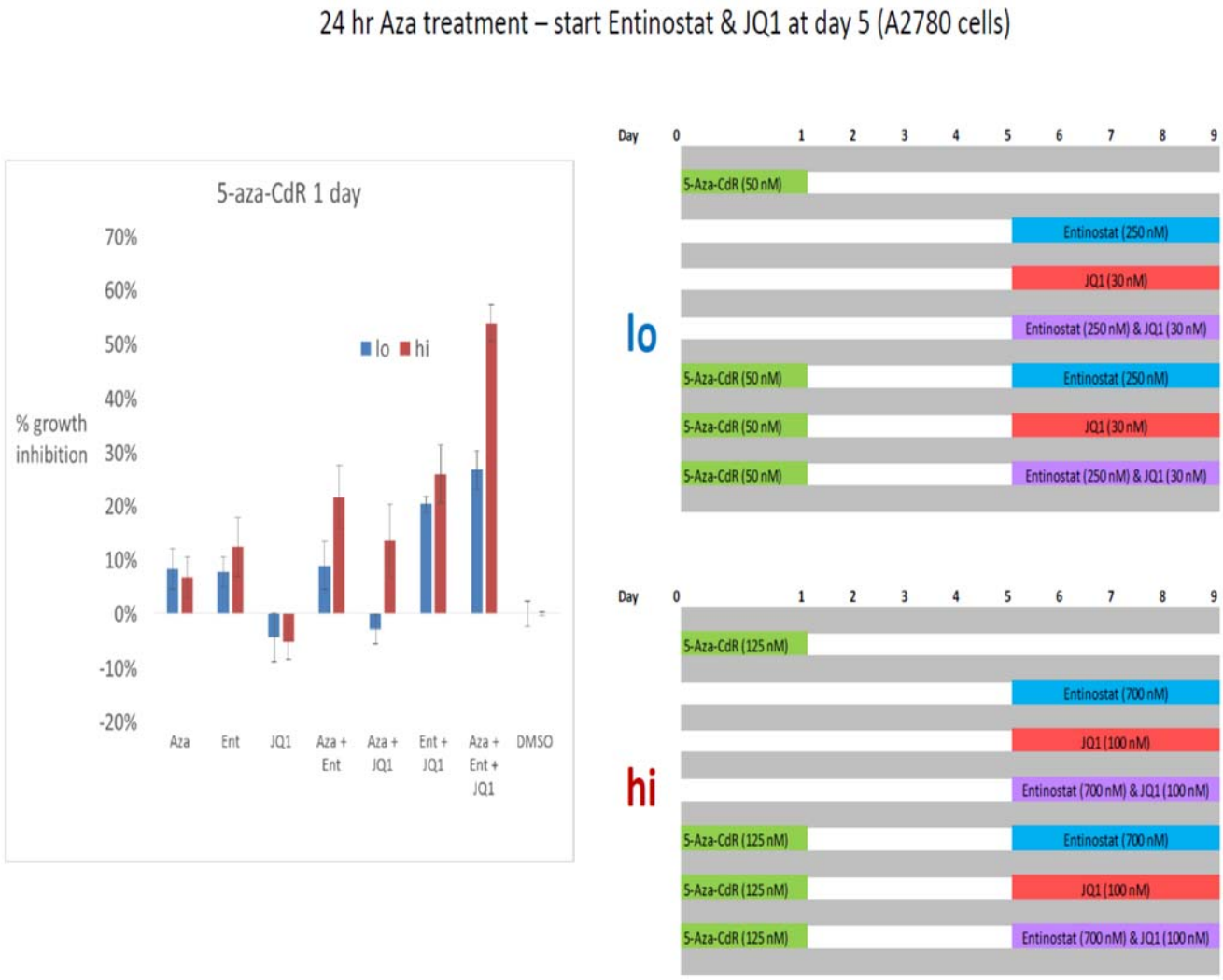
Fig. 10. Effects of in-vivo treatment with the schema in Fig. 7 of AZA and/or entinostat (MS275) and/or givinostat (ITF) on activation of CD8 T-cells (to become helper cells – left panel) and CD4 T-cells (to become effector cells – right panel) as measured by positivity for IFN gamma (Y-axis for percent positive cells).

C. Summary of progress for Specific Aim 3 and majors tasks 1 and 2: As outlined in the sections above, we have made exciting progress in defining mechanisms which could play a major role in using epigenetic therapy to sensitize patients with OC to immune checkpoint therapy. We have demonstrated, and published in Cell, the novel insight that AZA up-regulation of a double stranded RNA (dsRNA) viral defense pathway, and a role for ERV transcripts in this process, could be a core part of this possibility. We have evolved a strategy for augmentation of these AZA effects by combining this drug with HDACi's. From this, we developed a new, chronic low dose in vivo treatment schedule of these latter drugs in combination with AZA and shown that this can, in combination with immune checkpoint therapy, increase tumor signaling for attraction of activated T-cells and provide for anti-tumor effects with prolongation of survival. These data provide substrate for our honing this treatment regimen further during the coming year, understanding more about the effects on host immune cells, and moving the implications of these studies towards clinical trial development for patients with OC.

D. Specific Aim 4: *Develop new combinations of epigenetic drugs which may provide for the highest level of efficacy for management of OC***: Major Task 1:** *Follow biochemical hypotheses for designing combinations of the epigenetic drugs used in all studies above with new agents targeting additional steps in chromatin control of gene expression – the goal is to improve reversal of abnormal*

gene silencing in OC: These studies, under the direction of Dr. Peter Jones, have been initiated during the first year. The first paradigm investigated is an attempt to develop a next combinatorial epigenetic combination approach combining AZA and HDACi's with the BRD4 inhibitor, JQ1. BRD4 is a member of the bromodomain family which recognizes acetylated lysines on histones and especially those which facilitate activity of the c-MYC oncogene. JQ1 derivatives are now in early clinical trials for leukemias and lymphomas and blocking c-MYC is thought to be a major mechanism underlying their potential efficacy. Our hypothesis is that we can enhance anti-tumor effects because AZA and HDACi's may reactivate tumor suppressor pathways while JQ1 simultaneously blocks the MYC oncogenic activity.

The first results have been obtained using low doses of the drugs alone and in combination to assess the effects on cell cytotoxicity. Some preliminary activity has been observed which indicates that by combining the three drugs, with AZA used for 1 or 5 days prior, JQ1 is converted from a non-cytotoxic drug in this setting to one that augments cytotoxicity when combined with AZA and the HDACi, entinostat (**Fig. 11**).



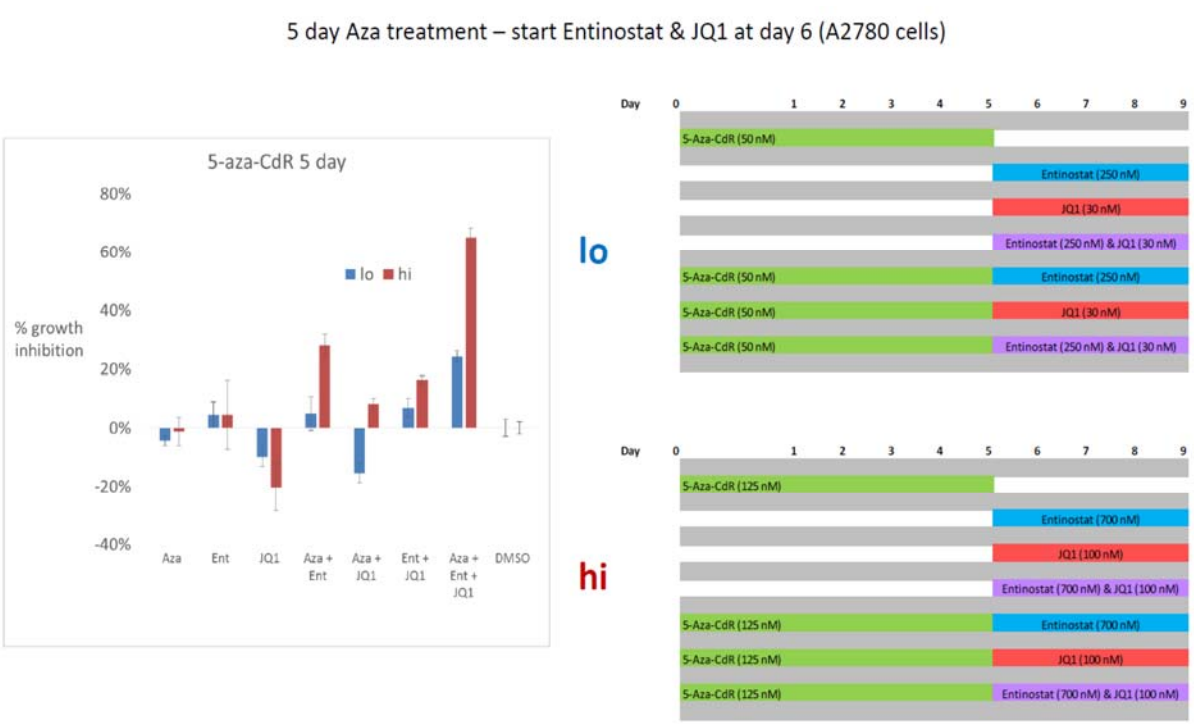


Fig. 11. Effects of in-vitro treatment with the schemas shown of deoxy-aza-cytidine (5-aza-CdR), entinostat, and JQ1. Note the enhanced growth inhibition (Y-axis) and red bars with the triple combination, after both 1 (upper panel) and 5 days (lower panel) of 5-aza-CdR.

We will be building on these results during the present year.

What opportunities for training and professional development did the project provide?

The first year has been exceptionally important in this regard. Dr. Chiappinelli, our mentored fellow trainee, has particularly benefitted from the work and become a real leader in multiple projects as per her accomplishments discussed extensively in the sections above (**Figs. 1-4**). Her academic growth is discussed in detail in Section 8, Special Reporting, later below.

A graduate student, Meredith Stone, has also benefitted tremendously from principally working with TEAL faculty investigator, Cindy Zahnow. She is centering her thesis work on the mouse model introduced in sections earlier and will be the first author for reports which will result from the studies outlined in **Figs. 5-10** in the progress report. She has just given an oral presentation on these studies at the AACR conference on OC in Oct. 2015.

How were the results disseminated to communities of interest?

As outlined in multiple sections below, the studies during the past year have resulted in faculty and trainees supported by the TEAL presenting and participating in many research forums for OC research, in specific, and cancer research in general. In addition, the recent Cell paper (included in Appendix), described in the Progress Report above, has generated great interest in our work and its implications for the therapy of OC.

What do you plan to do during the next reporting period to accomplish the goals and objectives?

During the next year we are hopeful to continue the same pace and volume of work as during this past one. We are generating a manuscript on the data in Figs. 5-10 which we hope to submit by the

end of 2015 and we will continue attending the many meetings which are highlighted in the sections below.

4. IMPACT

***What was the impact on the development of the principal discipline(s) of the project*?**

The work in the past year has made a big impact on the OC, and entire cancer research community for exhibiting the potential for epigenetic therapy to sensitize to immune checkpoint therapy and for providing insight into the mechanisms that may be involved. As per sections above, the trainees and faculty involved with the TEAL have been called upon for many lectures at OC specialty meetings and general cancer research meetings. The molecular studies, especially those in the recent Cell paper, have provided the nidus for the correlative science studies in the upcoming trial with Celgene and Merck to test whether AZA therapy can sensitize to anti-PD1 therapy for patients with advanced OC. This will involve using expression of the viral defense gene panel and ERV's to determine whether this predicts and tracks with therapy efficacy.

***What was the impact on other disciplines*?**

Our studies of OC outlined in the Progress Report have great implications for cancer in general. The AZA induced viral defense signature and ERV up-regulation can be seen for colon cancer and NSCLC and gene expression subgroups for potential predictive value are observed in breast, colon, melanoma, NSCLC as well as OC in TCGA data as reported in our recent Cell paper. In addition to the relevance for cancer, the studies outlined in Figs. 1-10 have much to teach about immunology as it pertains to cancer.

***What was the impact on technology transfer*?**

The entire AIM signature, inclusive of the viral defense gene signature and ERV transcripts are the subject of a patent applied for concerning their use as biomarker systems to predict and monitor the efficacy of applying epigenetic therapy to sensitize patients with advanced OC, and all cancer types, to immune checkpoint therapy.

***What was the impact on society beyond science and technology*?**

Hopefully the biggest impact of our studies will be for patients. A trial for testing our paradigm for sensitizing to immune checkpoint therapy in patients with advanced OC is scheduled to start by the first quarter of next year. Hopefully, efficacies observed in this trial will provide the greatest impact we could seek for our work.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

At present, we do not anticipate any major changes to our work scope and directions. We will continue the emphasis on Specific Aim 3, as focused upon in this progress report. This will involve trying to maximize a therapy strategy in our mouse OC model employing optimal timing and dosage for the combination of AZA plus the HDACis, entinostat and givinostat plus anti-PD-1. We will also continue work in Specific Aims 1 to test the direct anti-tumor effects, alone, of our combinatorial epigenetic therapy with AZA plus HDACis on OC in the in-vitro and in-vivo systems we are evolving. We will also pursue the ongoing work with new drug combinations in Specific Aim 4. This includes deciphering mechanisms and pathways involved with any efficacies seen.

Actual or anticipated problems or delays and actions or plans to resolve them

None anticipated at this time.

Changes that had a significant impact on expenditures

None anticipated at this time.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

None anticipated at this time.

6. PRODUCTS

A patent has been filed for use of the AIM gene panel, and most specifically expression of the viral defense genes and the ERV's as a biomarker system to potentially predict which patients with advanced OC and other cancers may respond to immune checkpoint therapy alone, and which might best benefit from prior administration of epigenetic therapy.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Individuals who have worked on the project

Johns Hopkins University

Name:	Stephen B. Baylin, M.D.
Project Role:	PI (Senior/Key Personnel)
Research Identifier:	N/A
Nearest person month worked:	4
Contribution to Project:	Dr. Baylin oversees all studies and activities conducted under this proposal.
Funding Support:	See Other Support

Name:	Cynthia Zahnow, Ph.D.
Project Role:	Co-Investigator (Senior/Key Personnel)
Research Identifier:	N/A
Nearest person month worked:	2
Contribution to Project:	Dr. Zahnow collaborates with Dr. Baylin on all of the studies in the lab.
Funding Support:	See Other Support

Name:	Drew Pardoll, M.D., Ph.D.
Project Role:	Co-Investigator (Senior/Key Personnel)
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Pardoll works with the Baylin group for all of the studies on how epigenetic therapy can sensitize ovarian cancers to immune checkpoint therapy.
Funding Support:	See Other Support

Name:	Ray-Whay Yen
Project Role:	Research Associate
Research Identifier:	N/A
Nearest person month worked:	8
Contribution to Project:	Ms. Yen is responsible for working with the entire Hopkins group for all of the pre-clinical work on ovarian cancer.
Funding Support:	No change

Name:	Katherine Chiappinelli, Ph.D.
Project Role:	Postdoctoral Fellow / Teal Junior Scientist
Research Identifier:	N/A
Nearest person month worked:	0 (salary is covered by F32 CA183214)
Contribution to Project:	Dr. Chiappinelli has become a real leader in multiple projects as per her accomplishments discussed extensively in the sections above. Her academic growth is discussed in detail in Section 8, Special Reporting.
Funding Support:	No change

Name:	Meredith Stone
Project Role:	Graduate Student
Research Identifier:	N/A
Nearest person month worked:	9
Contribution to Project:	Ms. Stone works along with Dr. Zahnow.
Funding Support:	No change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yes. See next pages for Drs. Baylin, Zahnow and Pardoll's Other Support.

OTHER SUPPORT

BAYLIN, STEPHEN B.

CURRENT

P50 CA058184 (PI: Baylin)
Title: SPORE in Lung Cancer (Project 1)
Time Commitment: 0.36 calendar
Supporting Agency: NIH/NCI
Procuring Contracting/Grants Officer: Peter Ujhazy
Address of Grants Officer: National Cancer Institute, Building 6116, 6116 Executive Blvd, Rockville, MD 20852
Performance Period: 9/5/1997-11/30/2015
Level of Funding: $242,211 (NCE)
Project's Goal(s): This project involves DNA methylation changes in cancer concerned with their translational implications for lung neoplasms.
Specific Aims: 1. To determine if novel biomarkers added to the current gene panel can enhance the predictive index, for tumor recurrence and death, of a DNA hypermethylation marker system for re-staging of stage I NSCLC. 2. To validate prospectively our findings that changes in promoter DNA methylation can molecularly restage stage I to stage III lung NSCLC and predict early disease recurrence and death. 3. To determine in a prospective controlled clinical trial of stage I NSCLC cancer patients, whether adjuvant epigenetic therapy improves overall and disease-free survival.
Project Overlap or Parallel: No scientific or budgetary overlap.

P50 CA058184 (PI: Baylin)
Title: SPORE in Lung Cancer (Administrative Core)
Time Commitment: 0.36 calendar
Supporting Agency: NIH/NCI
Procuring Contracting/Grants Officer: Peter Ujhazy
Address of Grants Officer: National Cancer Institute, Bldg. 6116-7109, 6116 Executive Blvd, Rockville, MD 20852
Performance Period: 9/5/1997-11/30/2015
Level of Funding: $80,250 (NCE)
Project's Goal(s): This project involves DNA methylation changes in cancer concerned with their translational implications for lung neoplasms.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

P30 CA006973 (PI: Nelson)
Title: Regional Oncology Research Center – Senior Leader
Time Commitment: 0.6 calendar
Supporting Agency: NIH/NCI
Procuring Contracting/Grants Officer: Devi Vembu
Address of Grants Officer: National Cancer Institute, Building 6116-700, 6116 Executive Blvd, Rockville, MD 20852
Performance Period: 5/7/1997-4/30/2017
Level of Funding: $12,327 (salary support only)
Project's Goal(s): CORE grant for the Johns Hopkins Oncology Center. Stephen Baylin receives salary support only for leadership and microarray core responsibilities.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

R01 ES011858 (PI: Baylin)
Title: DNA Methyltransferase Gene Expression in Colon Cancer
Time Commitment: 0.6 calendar
Supporting Agency: NIH/NIEHS

Procuring Contracting/Grants Officer: Frederick Tyson
Address of Grants Officer: National Institute of Health, Keystone Park 3064, 615 Davis Dr, Durham, NC 27709
Performance Period: 4/1/1991-5/31/2019
Level of Funding: $270,498
Project's Goal(s): Understand, further, the role of altered regulation and patterns of DNA methylation in the progression of colon cancer.
Specific Aims: 1. To determine mechanisms by which SOX17 blocks Wnt activation in CRC evolution. 2. To develop mouse models for CRC evolution based on epigenetic loss of Hic1. 3. To explore specific stages of CRC tumorigenesis mediated by epigenetic silencing of stem/progenitor cell related genes. 4. To define molecular determinants which initiate and/or maintain gene promoter DNA hypermethylation and gene silencing in CRC evolution.
Project Overlap or Parallel: No scientific or budgetary overlap.

U01 HL099775 (PI: Friedman)
Title: Basic and Translational Research of iPSC-Based Hematologic & Vascular Therapies – Project 2
Time Commitment: 0.46 calendar
Supporting Agency: NIH/NHLBI
Procuring Contracting/Grants Officer: Denis Buxton
Address of Grants Officer: NHLBI, Two Rockledge Center, Suite 8216, 6701 Rockledge Dr., Bethesda, MD 20817
Performance Period: 9/30/2009-4/30/2016
Level of Funding: $245,000
Project's Goal(s): This work will provide basic insight into how stem cells can be generated from adult cells and how these cells can be directed to develop into blood cells or blood vessel cells to benefit patients with hematologic or vascular disorders.
Specific Aims: 1. Precisely characterize the degree of cellular transformation observed at early stages of iPSC generation that are caused by reprogramming factor-mediated epigenetic changes, and the role that various factors, and protocols for introducing these factors, play in eliciting these alterations. 2. Determine the molecular mechanisms that induce abnormal epigenetic events during iPSC generation. 3. Determine whether refining use of DAC and TSA during iPSC generation, together with manipulation of DNMTs or other members of the repressive complex, can increase the efficiency of obtaining iPSC, while maximally reducing their tumorigenic potential and enhancing their regeneration potential.
Project Overlap or Parallel: No scientific or budgetary overlap.

90046519 (PI: Baylin/Casero/Zahnow)
Title: Novel Therapies Targeting Epigenetic Silencing of Tumor Suppressors
Time Commitment: 0.12 calendar
Supporting Agency: Samuel Waxman Cancer Research Foundation
Procuring Contracting/Grants Officer: Carole Asher
Address of Grants Officer: 420 Lexington Ave., Suite 825, New York, NY 10170
Performance Period: 7/1/2011-6/30/2016
Level of Funding: $100,000
Project's Goal(s): The goals of this project are: Project 1: To examine newly identified lysine specific demethylase 1 (LSD1) inhibitors in order to advance the understanding of the functioning and targeting of LSD1 for clinical utility. Project 2: To show that epigenetic therapy at very low, non-toxic doses, can dramatically blunt the tumorigenic properties of subpopulations of leukemic and solid tumor populations of "stem-like" cells. Project 3: To demonstrate that low dose epigenetic therapy re-sensitizes drug tolerant breast cancer cells to conventional, single agent chemotherapeutics or targeted therapy.
Specific Aims: 1. To perform, in Kasumi AML cells, and other lines, genome-wide studies of DNA methylation, chromatin and, gene expression patterns, including pathway analyses, for activating and repressive marks in separated populations of tumorigenic CD34+/CD38- versus non-tumorigenic CD34- cells. 2. To examine changes in the above genome-wide patterns induced by low doses of DNA

demethylating and histone deacetylation inhibiting drugs, already shown to inhibit the leukemic engraftment of the whole cell population, alone and together, on the above separated populations. 3. To derive markers for prediction and monitoring of epigenetic therapy from the above studies and which can be studied in primary tumor samples, and patient samples.
Project Overlap or Parallel: No scientific or budgetary overlap.

R01 CA170550 (PI: Laird/Jones)
Title: Epigenetic Drivers of Cancer
Time Commitment: 0.6 calendar
Supporting Agency: University of Southern California
Procuring Grants Officer: Emily Greenspan
Address of Grants Officer: 31 Center Drive, Room 10A-33, Bethesda, MD 20892
Performance Period: 9/1/2012-6/30/2016
Level of Funding: $130,119
Project Goals: We propose to address PQ10: As we improve methods to identify epigenetic changes that occur during tumor development, can we develop approaches to discriminate between "driver" and "passenger" epigenetic events?
Specific Aims: 1. To develop a probabilistic framework for predicting and prioritizing candidate epigenetic driver loci. 2. To select candidate epigenetic drivers of colon, breast, and lung cancer. 3. To functionally test candidate epigenetic drivers of colon, breast, and lung cancer.
Project Overlap or Parallel: No scientific or budgetary overlap.

90052001 (PI: Baylin)
Title: Bringing Epigenetic Therapy to the Management of Ovarian and Other Cancers
Time Commitment: 1.2 calendar
Supporting Agency: Miriam & Sheldon Adelson Medical Research Foundation
Procuring Contracting/Grants Officer: Joseph Bigley
Address of Grants Officer: OncoMethylome Sciences, 2505 Meridian Parkway, Suite 310, Durham, NC 27713
Performance Period: 10/1/2014-9/30/2016
Level of Funding: $436,619
Project's Goal(s): We are embarked on in-depth pre-clinical studies designed to directly bring "epigenetic" therapy, using existing DNA de-methylating agents and histone deactylase inhibitors (HDACi's), to the therapeutic management of advanced ovarian and other cancers.
Specific Aims: N/A
Project Overlap or Parallel: While both of these projects are aimed at taking novel approaches to radically improving the management of women with ovarian cancer, and can inform one another, they fund separate activities vital to this quest. First, while both grants seek to use information to leverage key clinical trials for ovarian cancer, only the Adelson supports activities for implementation of these trials, the bulk of biopsy acquisitions, and any other partial trial costs not otherwise covered. Second, many aspects for studies of host immune cell responses to epigenetic drugs are covered only in the Adelson funding, including work and support of collaborators, and genomics studies, in this effort, while the TEAL funds primarily work for response of ovarian cancer cells. Third, Only the TEAL award supports the efforts of the junior investigator mentored by Dr. Baylin to dissect how increased expression of endogenous viruses induced by epigenetic agents elicit up-regulation of ovarian cancer cell viral defense pathways. Lastly, only the Adelson supports work to use epigenetic agents to sensitize ovarian cancer to PARP inhibitors and work, with other collaborators for bring novel epigenetic therapy agents to treatment of ovarian cancer. However, this information can all be brought to bear on work in the TEAL in later years of the grant if indicated.

W81XWH-13-1-0199 (PI: Chan/Baylin)
Title: Targeting Master Regulators of the Breast Cancer Metastasis Transcriptome
Time Commitment: 0.24 calendar

Supporting Agency: Memorial Sloan-Kettering Cancer Center
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 7/1/2013-6/30/2018
Level of Funding: $61,555
Project's Goal(s): The Baylin lab will help perform CHIP seq and help analyze the chromatin state data for both the isogenic cell line systems that model differential metastatic ability.
Specific Aims: Aim 1: N/A
Justification: This grant has no overlap with the current proposal.
Project Overlap or Parallel: No scientific or budgetary overlap.

Award ID: W81XWH-14-1-0385(Baylin)
Title: A New Paradigm for the Treatment of Ovarian Cancer: The Use of Epigenetic Therapy to Sensitize Patients to Immunotherapy and Chemotherapy
Effort: 4.2 calendar
Supporting Agency: CDMRP
Name of Procuring Contracting/Grants Officer: Susan Dellinger, Grants Officer
Address of Funding Agency: 1077 Patchel St., Bldg 1077, Fort Detrick, MD 21702
Period of Performance: 09/30/2014-09/29/2019
Level of Funding: $436,208 annual direct costs
Project's Goal: The major goal of this project is to robustly prolong the survival of patients with serous ovarian cancer (OC) through introducing epigenetic therapy paradigms
Specific Aims: 1) To uncover the mechanisms through which epigenetic therapy may, alone, achieve robust, durable responses in patients with advanced ovarian cancer (OC), 2) Study how epigenetic therapy may sensitize OC cells to subsequent chemotherapies, 3) Study how epigenetic therapy may sensitize OC cells to subsequent immunotherapies which targets checkpoints which are driving immune tolerance, 4) Develop new combinations of epigenetic drugs which may provide for the highest level of efficacy for management of OC, 5) Bring all of the above studies to bear on leveraging clinical trials of epigenetic therapy on OC.
Role: PI
Overlap: None

AWARDED SINCE LAST SUBMISSION

R01 CA185357 (PI: Ahuja)
Title: (PQD3) Molecular Profiles associated with Long-Term Survival in pancreas Cancer
Time Commitment: 0.24 calendar
Supporting Agency: NCI
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 4/1/2014-3/31/2018
Level of Funding: $341,620
Project's Goal(s): Identify genomic and epigenomic signatures of pancreas cancer patients who have long-term survival using a large dataset.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

(PI: Baylin)
Title: The intersection of epigenetic and immune checkpoint therapy
Time Commitment: 0.12 calendar
Supporting Agency: AACR – Phillip A. Sharp Innovation in Collaboration Award
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 7/1/2014-12/31/2015 (NCE)

Level of Funding: $227,275
Project's Goal(s): Utilize results from all studies to help craft leveraged clinical trials for lung, melanoma and other cancers which are based on hypotheses derived from the data.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

(PI: Baylin)
Title: Clinical trials of epigenetic therapy sensitized patients with advanced non-small cell lung cancer to chemotherapy and immunotherapy
Time Commitment: 0.12 calendar
Supporting Agency: AACR – Jim Toth Sr. Breakthrough Prize in Lung Cancer
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 7/1/2014-6/30/2016
Level of Funding: $340,380
Project's Goal(s): N/A
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

(PI: Baylin)
Title: Clinical trials of epigenetic therapy in non-small cell lung cancer
Time Commitment: 0.3 calendar
Supporting Agency: Rising Tide Foundation
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 1/1/2015-12/31/2018
Level of Funding: $331,360
Project's Goal(s): We are addressing the hypothesis that reversal of cancer-specific DNA methylation and chromatin abnormalities can potently change the management of NSCLC.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

(PI: Zambidis)
Title: Functional vascular progenitors from naïve human iPSC
Time Commitment: 0.36 calendar
Supporting Agency: NCI
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 5/1/2015-2/29/2020
Level of Funding: $207,500
Project's Goal(s): To develop novel gene targeting and regeneration approaches for treating pediatric and adult vascular disorders using a newly discovered class of human iPSC converted to a ground state of naïve pluripotency.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

(PI: Baylin)
Title: VARI-SU2C Epigenetics Dream Team
Time Commitment: 0.12 calendar
Supporting Agency: Van Andel Research Institute
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 10/1/2014-9/30/2017
Level of Funding: $190,909
Project's Goal(s): Our Dream Team unites scientists at major cancer research institutions who are poised to propel the early promise of epigenetic therapy in blood malignancies to the forefront of management for patients with breast, colon and lung cancer.

Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

(PI: Brahmer/Baylin)
Title: Viral Defense Gene Expression Patterns and Response to Immune Checkpoint Blockade in NSCLC
Time Commitment: 0.24 calendar
Supporting Agency: Bristol-Myers Squibb
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 7/1/2015-6/30/2017
Level of Funding: $72,674
Project's Goal(s): This projects seeks to develop a biomarker to predict benefit from immunotherapy and to define if epigenetic modulation synergizes with immune checkpoint blockade.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

COMPLETED SINCE LAST SUBMISSION

R01 CA043318 (PI: Baylin)
Title: Methylation of the Calcitonin Gene in Human Tumors
Time Commitment: 0.36 calendar
Supporting Agency: NIH/NCI
Procuring Contracting/Grants Officer: Paul Okano
Address of Grants Officer: National Cancer Institute, Executive Plaza North, Suite 5024, 6130 Executive Blvd., Rockville, MD 20852
Performance Period: 9/30/1986-2/28/2015
Level of Funding: $117,255 (NCE)
Project's Goal(s): Study of the function of a new gene on chromosome 17p13.3 which is hypermethylated in human neoplasia, study of whether the estrogen receptor gene is a tumor suppressor in leukemia, and determination of whether overexpression of the DNA-methyltransferase gene mimics specific tumor suppressor gene inactivation events.
Specific Aims: 1. To explore how cancer specific, DNA hypermethylation of HIC1 may initiate an epigenetic network leading to tumor initiation. 2. To construct in vitro models to determine, precisely, what cellular steps in tumorigenesis can be initiated by epigenetically mediated loss of gene function. 3. To explore interactions between the polycomb group (PcG) of long term gene silencing proteins and SIRT1, DNA methyltransferases, and other key chromatin mediating proteins, which may link transcriptional repression in embryonic stem/progentitor cells and gene vulnerability to abnormal, promoter CpG island, DNA methylation in cancer
Project Overlap or Parallel: No scientific or budgetary overlap.

MDXHealth Incorporated (PI: Herman)
Title: Methylation-Specific PCR Technology (MSP) – Research Agreement
Time Commitment: 0.12 calendar
Supporting Agency: OMS
Procuring Contracting/Grants Officer: Joseph Bigley
Address of Grants Officer: OncoMethylome Sciences, 2505 Meridian Parkway, Suite 310, Durham, NC 27713
Performance Period: 7/1/2003-6/30/2014
Level of Funding: $50,473
Project's Goal(s): N/A
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

BioNumerik Pharmaceuticals, Inc. (PI: Baylin)
Title: Collaboration agreement with BioNumerik Pharmaceuticals, Inc.
Time Commitment: 0.12 calendar

Supporting Agency: BioNumerik
Procuring Contracting/Grants Officer: Frederick H. Hausheer, M.D.
Address of Grants Officer: BioNumerik Pharmaceuticals, Inc., 8122 Datapoint Drive, Suite 1250, San Antonio, TX 78229
Performance Period: 11/19/2001-2/09/2015
Level of Funding: $25,236
Project's Goal(s): The overall program will be aimed at the discovery and development of novel therapeutic agents that modulate DNA methylation in cancer. The focus will be to synthesize, patent and test novel chemical entities which target cancer cells with altered DNA methylation.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

U24 CA143882 (PI: Laird/Baylin)
Title: The USC-JHU Reference Epigenome Characterization Center
Time Commitment: 0.6 calendar
Supporting Agency: University of Southern California via NIH/NCI
Procuring Contracting/Grants Officer: Joseph Vockley
Address of Grants Officer: National Cancer Institute, Building 31 - Claude D Pepper Building, 3A20, 31 Center Dr.,
Bethesda, MD 20814
Performance Period: 9/29/2009-7/31/2014
Level of Funding: $133,867
Project's Goal(s): Genome Characterization Centers and Genome Data Analysis Center for the Cancer Genome Atlas Research Network (TCGA). The major goal of this project will be to genome wide measurement and characterization of patterns of DNA methylation in human cancers.
Specific Aims: 1. To characterize the DNA methylation status of 27,578 CpG dinucleotides located in 14,495 gene promoters in at least 10,000 human cancer samples and 1,000 control samples using the lllumina Infinium DNA Methylation analysis platform. 2. To transition epigenomic data production in TCGA to whole genome shotgun bisulfite sequence analysis to provide single-base-pair resolution DNA methylation data for TCGA cancer samples**.** 3. To implement quality control and quality assurance measures to ensure that epigenomic data deposited for public dissemination meets rigorous standards
Project Overlap or Parallel: No scientific or budgetary overlap.

SU2C-AACR-CT0109 (PI: Baylin/Jones)
Title: Bringing Epigenetic Therapy to the Forefront of Cancer Management
Time Commitment: 0.6 calendar
Supporting Agency: American Association for Cancer Research
Procuring Contracting/Grants Officer: Dr. Kimberly Sabelko
Address of Grants Officer: AACR, 625 Chestnut Street, 17th Floor, Philadelphia, PA 19106
Performance Period: 12/1/2009-1/31/2015
Level of Funding: $2,811,168 (NCE)
Project's Goal(s): Our Dream Team unites scientists at five major cancer research institutions who are poised to propel the early promise of epigenetic therapy in blood malignancies to the forefront of management for patients with breast, colon, and lung cancer.
Specific Aims: 1. To develop molecular markers which predict, and monitor, the efficacy of cancer epigenetic therapies. 2. To perform clinical trials to bring epigenetic therapy to the forefront of cancer management. 3. To determine whether a key mechanism for efficacy of epigenetic therapy is targeting and exhaustion of self-renewing cancer cells. 4. To develop a clinical trial with a new drug designed to circumvent the instability of 5-AC and DAC. 5. To determine targets in addition to promoter DNA hypermethylation that may be utilized in new cancer epigenetic therapy approaches.
Project Overlap or Parallel: No scientific or budgetary overlap.

U54 CA151838 (PI: Searson)
Title: Center of Cancer Nanotechnology Excellence at Johns Hopkins
Time Commitment: 0.6 calendar
Supporting Agency: NIH/NCI
Procuring Contracting/Grants Officer:
Address of Grants Officer:
Performance Period: 8/25/2010-7/31/2015
Level of Funding: $12,175 (Project 1 – salary support only)
Project's Goal(s): The goal of this Center is to integrate nanotechnology-based diagnostic and therapeutic tools for comprehensive cancer care.
Specific Aims: 1. To develop an integrated sample preparation method combining DNA isolation and bisulfate conversion into a single tube process. 2. To develop a highly sensitive technology enables by convergence of QD-FRET and MSP for detection of DNA methylation. 3. To develop a droplet microfluidic platform for fully integrated sample preparation and QD-FRET sensing, facilitating robust and high-throughput screening of DNA methylation. 4. To evaluate the new methylation screening platform and determine the potential use in early cancer detection and post-therapy monitoring.
Project Overlap or Parallel: No scientific or budgetary overlap.

90052339 (PI: Baylin)
Title: Epigenetic Therapy Sensitizing Breast Cancer to Blockades of DNA Repair
Time Commitment: 0.12 calendar
Supporting Agency: EIF – Entertainment Industry Foundation
Procuring Contracting/Grants Officer: Craig Cichy
Address of Grants Officer: 1201 West 5th Street, Ste T-700, Los Angeles, CA 90017
Performance Period: 10/15/2012-10/15/2014
Level of Funding: $125,000
Project's Goal(s): To understand how epigenetic therapy may regulate DNA repair and thereby sensitize breast cancer cells to therapeutic agent such as PARP inhibitors.
Specific Aims: N/A
Justification: This grant has no overlap with the current proposal.
Project Overlap or Parallel: No scientific or budgetary overlap.

SU2C-AACR-SF1-DT0109 (PI: Baylin/Jones)
Title: Bringing Epigenetic Therapy to the Forefront of Cancer Management
Time Commitment: 0.6 calendar
Supporting Agency: American Association for Cancer Research
Procuring Contracting/Grants Officer: Dr. Kimberly Sabelko
Address of Grants Officer: AACR, 625 Chestnut St., 17th Floor, Philadelphia, PA 19106
Performance Period: 12/01/2012 – 1/31/2015
Level of Funding: $1,100,000 (NCE)
Project's Goal(s): Our Dream Team unites scientists at five major cancer research institutions who are poised to propel the early promise of epigenetic therapy in blood malignancies to the forefront of management for patients with breast, colon, and lung cancer.
Specific Aims: 1) To develop molecular markers which predict, and monitor, the efficacy of cancer epigenetic therapy. 2) To perform clinical trials to bring epigenetic therapy to the forefront of cancer management. 3) To determine whether a key mechanism for efficacy of epigenetic therapy is targeting and exhaustion of self-renewing cancer cells. 4) To develop a clinical trial with a new drug designed to circumvent the instability of 5-AC and DAC. 5) To determine targets in addition to promoter DNA hypermethylation that may be utilized in new cancer epigenetic therapy approaches.
Justification: This grant has no overlap with the current proposal.
Project Overlap or Parallel: No scientific or budgetary overlap.

U24 CA143882 (PI: Laird/Baylin)
Title: The USC-JHU Reference Epigenome Characterization Center
Time Commitment: 0.12 calendar
Supporting Agency: University of Southern California via NIH/NCI
Procuring Contracting/Grants Officer: Joseph Vockley
Address of Grants Officer: National Cancer Institute, Building 31 - Claude D Pepper Building, 3A20, 31 Center Dr.,
Bethesda, MD 20814
Performance Period: 8/1/2013-7/31/2014
Level of Funding: $148,544 (Supplement)
Project's Goal(s): Genome Characterization Centers and Genome Data Analysis Center for the Cancer Genome Atlas Research Network (TCGA). The major goal of this project will be to genome wide measurement and characterization of patterns of DNA methylation in human cancers.
Specific Aims: 1. To characterize the DNA methylation status of 27,578 CpG dinucleotides located in 14,495 gene promoters in at least 10,000 human cancer samples and 1,000 control samples using the lllumina Infinium DNA Methylation analysis platform. 2. To transition epigenomic data production in TCGA to whole genome shotgun bisulfite sequence analysis to provide single-base-pair resolution DNA methylation data for TCGA cancer samples**.** 3. To implement quality control and quality assurance measures to ensure that epigenomic data deposited for public dissemination meets rigorous standards
Project Overlap or Parallel: No scientific or budgetary overlap.

OTHER SUPPORT

ZAHNOW, CYNTHIA A.

ACTIVE

90046519 (PI: Casero/Baylin/Zahnow)
Title: Novel therapies targeting epigenetic silencing of tumor suppressors
Time Commitment: .12 calendar
Supporting Agency: Samuel Waxman Cancer Research Foundation
Procuring Contracting/Grants Officer: Carole Asher
Address of Grants Officer: 420 Lexington Ave., Suite 825, New York, NY 10170
Performance Period: 7/1/2011-6/30/2016
Level of Funding: $50,000
Project's Goal(s): The goals of Dr. Zahnow's project within this Collaborative Grant is to demonstrate that low dose epigenetic therapy re-sensitizes drug tolerant breast cancer cells to conventional, single agent chemotherapeutics or targeted therapy.
Specific Aims: 1. To test whether Azacytidine can sensitize endocrine-resistant breast cancers to anti-estrogen therapy. 2. To continue our investigation of the role of the immune system in the anti-tumorigenic response of breast cancer cells to epigenetic therapy with a special focus on interferon signaling and activation. **Justification:** This grant has no overlap with the current proposal.
Project Overlap or Parallel: No scientific or budgetary overlap.

P30 CA006973 (PI: Nelson)
Title: Regional Oncology Research Center – Resource Director
Time Commitment: 1.2 calendar
Supporting Agency: NIH/NCI
Procuring Contracting/Grants Officer: Devi Vembu
Address of Grants Officer: National Cancer Institute, Building 6116-700, 6116 Executive Blvd, Rockville, MD 20852
Performance Period: 5/7/1997-4/30/2017
Level of Funding: $51,810 (salary support only)
Project's Goal(s): CORE grant for the Johns Hopkins Oncology Center. Dr. Zahnow receives salary support only for serving as the Director of the Animal Facility and administrative duties to the Oncology Center.
Specific Aims: N/A
Justification: This grant has no overlap with the current proposal.
Project Overlap or Parallel: No scientific or budgetary overlap.

R01 CA170550 (PI: Laird/Jones)
Title: Epigenetic Drivers of Cancer
Time Commitment: 1.2 calendar
Supporting Agency: University of Southern California
Procuring Contracting/Grants Officer: Emily Greenspan
Address of Grants Officer: 31 Center Dr., Rm. 10A-33, Bethesda, MD 20892
Performance Period: 9/01/2012-6/30/2016
Level of Funding: $130,119
Project Goals: We propose to address PQ10: As we improve methods to identify epigenetic changes that occur during tumor development, can we develop approaches to discriminate between "driver" and "passenger" epigenetic events?
Specific Aims: 1) To develop a probabilistic framework for predicting and prioritizing candidate

epigenetic driver loci 2) To select candidate epigenetic drivers of colon, breast, and lung cancer. 3) To functionally test candidate epigenetic drivers of colon, breast, and lung cancer.
Justification: This grant has no overlap with the current proposal.
Project Overlap or Parallel: No scientific or budgetary overlap.

90052001 (PI: Baylin)
Title: Bringing Epigenetic Therapy to the Management of Ovarian and Other Cancers
Time Commitment: 2.4 calendar
Supporting Agency: Miriam & Sheldon Adelson Medical Research Foundation
Procuring Contracting/Grants Officer: Marissa White
Address of Grants Officer: 300 First Avenue, Suite 330, Needham, MA 02494
Performance Period: 10/01/2014-9/30/2016
Level of Funding: $431,895
Project Goals: We are embarked on in-depth pre-clinical studies designed to directly bring "epigenetic" therapy, using existing DNA de-methylating agents and histone deactylase inhibitors (HDACi's), to the therapeutic management of advanced ovarian and other cancers.
Specific Aims: N/A
Justification: This grant has no overlap with the current proposal.
Project Overlap or Parallel: While both of these projects are aimed at taking novel approaches to radically improving the management of women with ovarian cancer, and can inform one another, they fund separate activities vital to this quest. First, while both grants seek to use information to leverage key clinical trials for ovarian cancer, only the Adelson supports activities for implementation of these trials, the bulk of biopsy acquisitions, and any other partial trial costs not otherwise covered. Second, many aspects for studies of host immune cell responses to epigenetic drugs are covered only in the Adelson funding, including work and support of collaborators, and genomics studies, in this effort, while the TEAL funds primarily work for response of ovarian cancer cells. Third, Only the TEAL award supports the efforts of the junior investigator mentored by Dr. Baylin to dissect how increased expression of endogenous viruses induced by epigenetic agents elicit up-regulation of ovarian cancer cell viral defense pathways. Lastly, only the Adelson supports work to use epigenetic agents to sensitize ovarian cancer to PARP inhibitors and work, with other collaborators for bring novel epigenetic therapy agents to treatment of ovarian cancer. However, this information can all be brought to bear on work in the TEAL in later years of the grant if indicated.

AWARDED SINCE LAST SUBMISSION

(PI: Baylin)
Title: The intersection of epigenetic and immune checkpoint therapy
Time Commitment: 0.6 calendar
Supporting Agency: AACR – Phillip A. Sharp Innovation in Collaboration Award
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 7/1/2014-12/31/2015
Level of Funding: $227,275
Project's Goal(s): Utilize results from all studies to help craft leveraged clinical trials for lung, melanoma and other cancers which are based on hypotheses derived from the data.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

(PI: Baylin)
Title: Clinical trials of epigenetic therapy in non-small cell lung cancer
Time Commitment: 0.3 calendar

Supporting Agency: Rising Tide Foundation
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 1/1/2015-12/31/2018
Level of Funding: $331,360
Project's Goal(s): We are addressing the hypothesis that reversal of cancer-specific DNA methylation and chromatin abnormalities can potently change the management of NSCLC.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

(PI: Brahmer/Baylin)
Title: Viral Defense Gene Expression Patterns and Response to Immune Checkpoint Blockade in NSCLC
Time Commitment: 1.2 calendar
Supporting Agency: Bristol-Myers Squibb
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 7/1/2015-6/30/2017
Level of Funding: $72,674
Project's Goal(s): This project seeks to develop a biomarker to predict benefit from immunotherapy and to define if epigenetic modulation synergizes with immune checkpoint blockade.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

COMPLETED SINCE LAST SUBMISSION

90038725 (PI: Baylin)
Title: The Lee Jeans Translational Breast Cancer Research Program
Time Commitment: 0.6 calendar
Supporting Agency: EIF – Entertainment Industry Foundation
Procuring Contracting/Grants Officer: Bobby Fergerstrom
Address of Grants Officer: 1201 West 5th Street, Ste T-700, Los Angeles, CA 90017
Performance Period: 7/1/2009-6/30/2014
Level of Funding: $159,090 (NCE)
Project's Goal(s): This project aims to extrapolate basic mechanisms underlying the efficacy of epigenetic therapy in pre-leukemic and leukemic states to studies of breast cancer cells representing the various subtypes of this disease and to rapidly implement these findings into clinical trials.
Specific Aims: 1) To expand our analysis of how breast cancer cell lines and primary tissue from breast cancer patients respond to low doses of the DNA demethylating agent 5-azacitidine (Vidaza, 5-AC, AZA), and the histone deacetylase inhibitor MS-275 (Entinostat), alone and in combination. 2) To determine how Vidaza and/or Entinostat may be used to re-sensitize drug tolerant breast cancer cells to therapies they have become resistant to. 3) To determine which chemotherapy regimen is most effective when used in combination with Vidaza and Entinostat.
Justification: This grant has no overlap with the current proposal.
Project Overlap or Parallel: No scientific or budgetary overlap.

SU2C-AACR-SF1-DT0109 (PI: Baylin/Jones)
Title: Bringing Epigenetic Therapy to the Forefront of Cancer Management
Time Commitment: 0.6 calendar
Supporting Agency: American Association for Cancer Research
Procuring Contracting/Grants Officer: Dr. Kimberly Sabelko
Address of Grants Officer: AACR, 625 Chestnut St., 17th Floor, Philadelphia, PA 19106
Performance Period: 12/01/2012 – 1/31/2015

Level of Funding: $1,100,000 (NCE)
Project's Goal(s): Our Dream Team unites scientists at five major cancer research institutions who are poised to propel the early promise of epigenetic therapy in blood malignancies to the forefront of management for patients with breast, colon, and lung cancer.
Specific Aims: 1) To develop molecular markers which predict, and monitor, the efficacy of cancer epigenetic therapy. 2) To perform clinical trials to bring epigenetic therapy to the forefront of cancer management. 3) To determine whether a key mechanism for efficacy of epigenetic therapy is targeting and exhaustion of self-renewing cancer cells. 4) To develop a clinical trial with a new drug designed to circumvent the instability of 5-AC and DAC. 5) To determine targets in addition to promoter DNA hypermethylation that may be utilized in new cancer epigenetic therapy approaches.
Justification: This grant has no overlap with the current proposal.
Project Overlap or Parallel: No scientific or budgetary overlap.

90052339 (PI: Baylin)
Title: Epigenetic Therapy Sensitizing Breast Cancer to Blockades of DNA Repair
Time Commitment: 0.12 calendar
Supporting Agency: EIF – Entertainment Industry Foundation
Procuring Contracting/Grants Officer: Craig Cichy
Address of Grants Officer: 1201 West 5th Street, Ste T-700, Los Angeles, CA 90017
Performance Period: 10/15/2012-10/14/2014
Level of Funding: $125,000 (NCE)
Project's Goal(s): To understand how epigenetic therapy may regulate DNA repair and thereby sensitize breast cancer cells to therapeutic agent such as PARP inhibitors.
Specific Aims: N/A
Justification: This grant has no overlap with the current proposal.
Project Overlap or Parallel: No scientific or budgetary overlap.

OTHER SUPPORT

PARDOLL, DREW M.

ACTIVE

Award ID: P50CA098252 (Wu)
Title: SPORE in Cervical Cancer
Effort: 0.36 calendar months
Supporting Agency: NIH/NCI
Name of Procuring Contracting/Grants Officer: Jason Gill
Address of Funding Agency: 9609 Medical Center Drive, Rockville, MD 20850
Performance Period: 09/01/04 – 08/31/19
Level of Funding: $1,668,395 annual direct costs
Project's Goal: The development research program role is to identify and select pilot projects with potential for development into full- fledged translational research avenues, collaborations, and new methodologies for integration into other research projects based on the described review criteria.
Specific Aims: 1) Provide initiating funds for novel explorations related to cervical cancer. 2) Integrate the awardee into the SPORE community by participation in monthly meetings, group communications, and opportunities for expanded funding and for collaborations. 3) Review progress and recommend avenues for continuation of successful projects
Role: Co- Director, Developmental Research Program
Overlap: None

Award ID: P30CA06973 (Nelson)
Title: Regional Oncology Research
Center
Effort: 0.6 calendar months
Supporting Agency: NIH/NCI
Name of Procuring Contracting/Grants Officer: Jason Gill
Address of funding agency: 9609 Medical Center Drive, Rockville, MD 20850
Performance Period: 08/09/2012-04/30/2017
Level of Funding: $20,276*
Project's Goal: The major goal of this project is to support research programs and shared resources at the National Cancer Institute Designated Cancer Center. The central goal of the Cancer Immunology program is the development of new effective cancer immunotherapies that are based on understanding the molecular recognition and regulation.
Specific Aims: N/A
Role: Co-Program Leader for Cancer Immunology (*salary support only)
Overlap: None

Award ID: 90054364 (Pardoll)
Title: International Immuno-Oncology Network (IION) Resource Model
Effort: 1.2 calendar months
Supporting Agency: Bristol- Myers Squibb Co
Name of Procuring Contracting/Grants Officer: Les Enterline
Address of Funding Agency: Route 206 and Providence Line Road, Princeton, NJ 08543
Period of Performance: 05/07/2013-05/06/2017
Level of Funding: $486,987 annual direct cost

Project's Goal: The major goals of this project are to dissect the tumor immune microenvironment in the context of therapy with immune checkpoint blockade and to monitor tumor burden using circulating tumor DNA (ctDNA)
Specific Aims: 1.) Analyze immune-inhibitory networks in resected tumors employing 3 techniques for geographic localization: (i) IHC, (ii) amplified ISH, and (iii) qRT-PCR analysis of laser capture micro-dissected (LCM) regions of leukocytic infiltration. 2.) Complementary to the studies in 1, we will sort myeloid, lymphoid and cancer cells from freshly dissociated tumors in cases where enough tumor is available, allowing analysis by flow cytometry and mRNA profiling of cellular subsets for co-expression of inhibitory ligands, receptors and druggable metabolic enzymes. 3.) Using qRT-PCR, amplified ISH and multiplex ELISA, we will analyze the spectrum of cytokines within the tumor microenvironment.
Role: PI
Overlap: None

Award ID: 273686 (Pardoll)
Title: CTLA-4 and anti-PD1 blockade: Correlative assessments for discovery
Effort: .36 calendar months
Supporting Agency: Bristol-Myers Squibb Co
Name of Procuring Contracting/Grants Officer: Les Enterline
Address of Funding Agency: Route 206 and Providence Line Road, Princeton, NJ 08543
Period of Performance: 07/10/2013-07/09/2016
Level of Funding: $125,000 annual direct costs
Projects Goal: The major goal of this project is to help guide optimal therapeutic combinations of co-inhibitory agents, to identify novel therapeutic Immune modulatory targets, and to predict, understand and overcome disease resistance.
Specific Aims: 1. Characterize changes in tumor infiltrating lymphocytes (TILs) and circulating peripheral blood cells from serially acquired tumor and blood samples from patients with therapeutic response to ipi, nivo and combination therapy with comparison to non-responders. 2. Characterize changes in metastatic melanoma cells, myeloid cells and tumor aSSOCiated fibroblasts from tumor samples from patients with therapeutic response 10 ipi, nivo and combination therapy with comparison to non-responders. 3. Compare finding from pre- and post-therapy specimens from patients treated with ipi and nivo as single agents vs combination therapy to identify unique potential biomarkers predictive of therapeutic response to combinatorial blockade.
Role: PI
Overlap: Overlaps with grant below. This is an Academic-Industry sponsored award with MRA being the prime sponsor and BMS being the industry sponsor. Each sponsor provides 50% support for the project.

Award ID: 273686
Title: CTLA-4 and anti-PD1 blockade: Correlative assessments for discovery
Effort: .36 calendar months
Supporting Agency: Memorial Sloan Kettering Cancer Center (Prime sponsor, Melanoma Research Alliance)
Name of Procuring Contracting/Grants Officer: Richard K. Naum
Address of Funding Agency: 1275 York Ave, New York, New York 10065
Performance Period: 07/10/2013-07/09/2016
Level of Funding: $125,000 annual direct costs
Project's Goal: The major goal of this project is to help guide optimal therapeutic combinations of co-inhibitory agents, to identify novel therapeutic Immune modulatory targets, and to predict, understand and overcome disease resistance

Specific Aims: 1. Characterize changes in tumor infiltrating lymphocytes (TILs) and circulating peripheral blood cells from serially acquired tumor and blood samples from patients with therapeutic response to ipi, nivo and combination therapy with comparison to non-responders. 2. Characterize changes in metastatic melanoma cells, myeloid cells and tumor aSSOCiated fibroblasts from tumor samples from patients with therapeutic response 10 ipi, nivo and combination therapy with comparison to non-responders. 3. Compare finding from pre- and post-therapy specimens from patients treated with ipi and nivo as single agents vs combination therapy to identify unique potential biomarkers predictive of therapeutic response to combinatorial blockade.
Role: PI
Overlap: Overlaps with grant above. This is an Academic-Industry sponsored award with MRA being the prime sponsor and BMS being the industry sponsor. Each sponsor provides 50% support for the project.

Award ID: SU2C-AACR-DT10
Title: Immunologic Checkpoint Blockade and Adoptive Cell Transfer in Cancer Therapy
Effort: 2.4 calendar months (20% effort, 10% salary support)
Supporting Agency: MD Anderson Cancer Center -Prime Sponsor-AACR (SU2C)
Name of Procuring Contracting/Grants Officer: Melinda Cotten
Address of Funding Agency: 1515 Holcombe Blvd, Houston, TX 77030-4000
Performance Period:3/1/2013-02/28/2016
Level of Funding: $468,182 annual direct cost
Project's Goal: The major goal of this project is to 1.) develop an increased understanding of immune cells and pathways within the tumor microenvironment that contribute to tumor resistance vs. rejection, 2.) identify the antigenic targets of both T and B cells response to checkpoint blockade, including unique neoantigens that arise as a result of missense mutations in the tumors and 3.) develop rationale combinatorial treatment regiments.
Specific Aims: Aim 1: Interrogation of the immune responses within the tumor microenvironment before and after treatment with immune checkpoint blockade. Aim 2: Interrogation of the targets of T and B cell responses after checkpoint blockade. Aim 3: Development of combinatorial cancer therapies based on checkpoint blockade.
Role: PI
Overlap: None

Award ID: N/A (Pardoll)
Title: CTLA-4 and anti-PD1 blockade: Correlative assessments for discovery
Effort: .12 calendar months
Supporting Agency: Bristol-Myers Squibb Co
Name of Procuring Contracting/Grants Officer: Les Enterline
Address of Funding Agency: Route 206 and Providence Line Road, Princeton, NJ 08543
Period of Performance: 07/10/2013-07/09/2016
Level of Funding: $125,000 annual direct costs
Projects Goal: The major goal of this project is to help guide optimal therapeutic combinations of co-inhibitory agents, to identify novel therapeutic Immune modulatory targets, and to predict, understand and overcome disease resistance.
Specific Aims: 1. Characterize changes in tumor infiltrating lymphocytes (TILs) and circulating peripheral blood cells from serially acquired tumor and blood samples from patients with therapeutic response to ipi, nivo and combination therapy with comparison to non-responders. 2. Characterize changes in metastatic melanoma cells, myeloid cells and tumor aSSOCiated fibroblasts from tumor samples from patients with therapeutic response 10 ipi, nivo and combination therapy with comparison to non-responders. 3. Compare finding from pre- and post-

therapy specimens from patients treated with ipi and nivo as single agents vs combination therapy to identify unique potential biomarkers predictive of therapeutic response to combinatorial blockade.
Role: PI
Overlap: Overlaps with grant below. This is an Academic-Industry sponsored award with MRA being the prime sponsor and BMS being the industry sponsor. Each sponsor provides 50% support for the project.

Award ID: W81XWH-14-1-0385(Baylin)
Title: A New Paradigm for the Treatment of Ovarian Cancer: The Use of Epigenetic Therapy to Sensitize Patients to Immunotherapy and Chemotherapy
Effort: .36 cal months
Supporting Agency: CDMRP
Name of Procuring Contracting/Grants Officer: Susan Dellinger, Grants Officer
Address of Funding Agency: 1077 Patchel St., Bldg 1077, Fort Detrick, MD 21702
Period of Performance: 09/30/2014-09/29/2019
Level of Funding: $436,208 annual direct costs
Project's Goal: The major goal of this project is to robustly prolong the survival of patients with serous ovarian cancer (OC) through introducing epigenetic therapy paradigms
Specific Aims: 1) To uncover the mechanisms through which epigenetic therapy may, alone, achieve robust, durable responses in patients with advanced ovarian cancer (OC) 2) Study how epigenetic therapy may sensitize OC cells to subsequent chemotherapies 3) Study how epigenetic therapy may sensitize OC cells to subsequent immunotherapies which targets checkpoints which are driving immune tolerance 4) Develop new combinations of epigenetic drugs which may provide for the highest level of efficacy for management of OC
5) Bring all of the above studies to bear on leveraging clinical trials of epigenetic therapy on OC
Role: Co-Investigator
Overlap: None

AWARDED SINCE LAST SUBMISSION

Award ID: 90062513 (Pardoll)
Title: The role of neuritin/sema4 interactions to promote expansion and persistence of Tregs
Effort: 1.2 calendar Months
Supporting Agency: Potenza Therapeutics, Inc
Name of Procuring Contracting/Grants Officer: Daniel J. Hicklin, PhD
Address of Funding Agency: 1030 Massachusetts Avenue, Suite 210, Cambridge, MA 02138
Performance Period: 04/01/2015-03/31/2017
Level of Funding: $348,837 annual direct costs
Project goal: The major goal of this project is to further delineate the role of the neuritin/sema4 interaction in promoting the expansion and persistence of Tregs
Specific Aims: 1) Generate panel of monoclonal antibodies with high affinity for neuritin. 2) Define in vitro and in vivo activity of these antibodies, particularly regarding Treg maintenance and function. Benchmark against Neutitin KO. 3) Define in vitro and in vivo activity of peptides that block Neuritin- Seama4A/D interaction and recombinant Neuritin protein. 4) Confirming and mapping the interaction between neuritin and semaphoring 4D. 5) Generate and screen for blocking antibodies which disrupt the neuritin/sema4D interaction. 6) Examine the expression of neuritin on Tregs in human peripheral blood and tumor infiltrating leukocytes. 7) Pharmacology studies to examine the activity of mAbs targeting the neuritin/sema4 interaction in several murine models of cancer (alone or in combination with other immunotherapies)
Role: PI

Overlap: None

Award ID: 308121 (Vogelstein)
Title: Mutational Density and Response to Immunotherapy with Checkpoint Blockade
Effort: 1.2 Calendar months
Supporting Agency: Melanoma Research Alliance
Name of Procuring Contracting/Grants Officer: Laura Brockway-Lunardi, Ph.D.
Address of Funding Agency: 1101 New York Avenue, Suite 620, Washington, DC 20005
Period of Performance: 05/15/2014-05/14/2017
Level of Funding: $300,000 annual direct costs
Projects Goal: The goal of this project is to lay the groundwork for a biomarker to predict response to anti-PD-1 antibodies and also for the generation of personalized melanoma vaccines that could be used in combination with anti-PD-1 therapy.
Specific Aims: 1.) Determine whether overall mutational load in melanoma correlates with PD-1 ligand (PD-L1 and PD-L2) expression and clinical response to anti-PD-1 treatment. 2.) Using algorithms for HLA binding, proteasome processing and TAP transport, determine whether predicted mutation-derived neoepitopes correlate with PD-1 ligand expression and clinical response to anti-PD-1 treatment and 3.) In selected cases, analyze T cell responses to predicted *neoepitopes* from peripheral blood lymphocytes and compare them with responses to epitopes from index *shared* melanosomal and cancer-testes antigens.
Role: Co-investigator
Overlap: None

Award ID: BMSC192 (Topalian/Pardoll)
Title: Analysis of PD-1 Blockade in Virus-Associated Cancers
Effort: **.**24 Calendar months
Supporting Agency: Bristol Myers Squibb Co
Name of Procuring Contracting/Grants Officer: Les Enterline
Address of Funding Agency: Route 206 and Providence Line Road, Princeton, NJ 08543
Period of Performance: 11/01/2014-10/31/2015
Level of Funding: $168,042 annual direct costs
Projects Goal: The major goal of this project is to characterize immune cell types and the expression of immune-modulating molecules (PD-1, PD-L1 and others) in the microenvironment of select cancers associated with EBV or HPV
Specific Aims: N/A
Role: PI
Overlap: None

Award ID: N/A (Pardoll)
Title: Analysis of novel immunomodulatory ligands and receptors
Effort: .6 calendar months
Supporting Agency: Compugen Ltd.
Name of Procuring Contracting/Grants Officer: Anat Cohen-Dayag, Ph.D.
Address of Funding Agency: 72 Pichas Rosen St., Tel Aviv 69512, Israel
Period of Performance: 12/17/2014-11/30/2019
Level of Funding: $331,395 annual direct costs
Project's Goal: The major goal of this project is to study the immunobiology and cancer immunotherapy relevance of multiple novel gene products identified as potentially immunomodulatory
Specific Aims: 1) Determine in-house phage display vs conventional hybridoma depending on level of conservation of molecule across species. 2) Expression studies in mice and humans-define

target's expression tumor components of the TME, sorted cell populations, purified tumor infiltrates, myeloid and lymphocyte human-on selected targets. 3) In vitro testing of murine and human antibodies and Fc fusion molecules 4)Antibody/Recombinant Fc fusion experiments with emphasis on antibodies 5) Therapeutic synergy experiments
Role: PI
Overlap: None

Award ID: 305021 (Topalian)
Title: Crossroads of Genetic and Immunologic Heterogeneity of Melanoma Metastasis
Effort: .24 calendar months* effort, no salary
Supporting Agency: Melanoma Research Alliance
Name of Procuring Contracting/Grants Officer: Laura Brockway-Lunardi, Ph.D.
Address of Funding Agency: 1101 New York Avenue, Suite 620, Washington, DC 20005
Period of Performance: 05/15/2014-05/14/2017
Level of Funding: $75,000 annual direct costs
Project's Goal: The major goal of this project is to characterize interactions between heterogeneous genetic and immunological factors in melanoma, by studying primary and metastatic tumors obtained through a rapid autopsy program
Specific Aims: 1) Establish a rapid autopsy biospecimen bank of primary and metastatic melanoma lesions from 8-10 patients. 2) Characterize genetic features of tumor clonal evolution through space (anatomic location) and time (primary lesion to metastasis). 3) Explore the immunological heterogeneity of metastasis. 4) Correlate genetic and immunological signatures in order to understand factors driving tumor-induced immunosuppression and progression
Role: Co-Investigator
Overlap: None

Award ID: N/A (Pardoll)
Title: The role of neuritin/sema4 interactions to promote expansion and persistence of Tregs
Effort: 1.2 cal months
Supporting Agency: Potenza Therapeutics
Name of Procuring Contracting/Grants Officer: Daniel J. Hicklin, PhD
Address of Funding Agency: 1030 Massachusetts Avenue, Suite 210, Cambridge, MA 02138
Period of Performance: 04/01/2015-03/31/2017
Level of Funding: $348,837 annual direct costs
Project's Goal: The major goal of this project is to further delineate the role of the neuritin/sema4 interaction in promoting the expansion and persistence of Tregs
Specific Aims: 1) Generate panel of monoclonal antibodies with high affinity for neuritin 2) Define in vitro and in vivo activity of these antibodies, particularly regarding Treg maintenance and function. Benchmark against Neutitin KO. 3) Define in vitro and in vivo activity of peptides that block Neuritin- Seama4A/D interaction and recombinant Neuritin protein. 4) Confirming and mapping the interaction between neuritin and semaphoring 4D. 5) Generate and screen for blocking antibodies which disrupt the neuritin/sema4D interaction. 6) Examine the expression of neuritin on Tregs in human peripheral blood and tumor infiltrating leukocytes. 7) Pharmacology studies to examine the activity of mAbs targeting the neuritin/sema4 interaction in several murine models of cancer (alone or in combination with other immunotherapies)
Role: PI
Overlap: None

COMPLETED SINCE LAST SUBMISSION

Award ID: R01CA151393 (Pardoll; Sears contact PI)
Title: Enterotoxigenic Bacteroides Fragilis: A Bacterial Promoter of Colon Oncogenesis
Effort: 1.2 calendar months
Supporting Agency: NIH/NCI
Name of Procuring Contracting/Grants Officer: Connie Murphy
Address of Funding Agency: National Cancer Institute, 8490 Progress Drive, Frederick, MD 21701
Performance Period: 09/02/10 – 07/31/15
Level of Funding: $296,951 annual direct cost
Project's Goal: The major goal of this project is to focus on analysis of the overall colonic microbiome and seek to determine whether there are general microbiome signatures that correlate with defined colonic immune responses and are associated with human colon cancer and proximal and distal flanking normal tissue.
Specific Aims: 1. To analyze the association of ETBF (together with specific isotypes of BFT) and colon cancer. 2. To identify Stat3 activation in colon cancer and define its association with intratumoral immune responses, particularly Th17 responses
Role: Co-PI
Overlap: None

Award ID: R01AI089830 (Pardoll)
Title: The Role of EOS in Regulatory T-Cell Biology
Effort: 1.32 calendar months
Supporting Agency: NIH/NIAID
Name of Procuring Contracting/Grants Officer: Mildred J. Qualls
Address of Funding Agency: 6700B Rockledge Drive, Bethesda, MD 20817
Performance Period: 07/01/10 - 06/30/15
Level of Funding: $232,650 annual direct cost
Project's Goal: The major goal of this project is to study the role of EOS in Regulatory T-Cell Biology
Specific Aims: 1.) Elucidate the role of metabolic stimuli that affect NADH/NAD balance in Eos mediated gene silencing via the CtBP1 complex. Our preliminary data demonstrate that the Rossman domain in CtBP1 may endow Treg cells to sense metabolic cues via the NADH/NAD redox balance. This hypothesis will be explored by analyzing NADH/NAD ratios in Treg, using drugs that alter the NADH/NAD balance and testing mutants of CtBP1 that fail to bind NAD(H). 2.) Study the regulation of Eos expression in Treg cells by microRNAs. Our preliminary data suggest that iR17-92 regulates Eos levels via RNA interference. We will explore the role of this and other candidate microRNAs in Eos regulation in Treg. 3) Study the consequences of Eos deletion to Treg cell homeostasis, differentiation and adaptive Treg cell development. We will employ both siRNA knockdown and conditional knockout of Eos to explore its role in homeostasis, differentiation and capacity of both natural and adaptive Treg to modulate Th1, Th2 and Th17 responses using well established *in vivo* systems.
Role: PI
Overlap: None

Award ID: R01CA151325 (Sears)
Title: Mechanisms of TH17 Inflammation-Induced Colon Carcinogenesis
Effort: 1.2 calendar months
Support Agency: NIH/NCI
Name of Procuring Contracting/Grants Officer: Cammie La

Address of Funding Agency: 9609 Medical Center Drive, Rockville, MD 20850
Performance Period: 07/06/2010 – 04/30/2015
Level of Funding: $190,950 annual direct cost
Project's Goal: The major goal of this project is to study the immune and genetic mechanisms by which a newly recognized common human stool bacterium called exterotoxigenic Bacteroides fragilis triggers colon tumors in mice, providing new insights into how colon cancer develops and potential new approaches to colon cancer therapy.
Specific Aims: 1) Define the components of the colonic Stat3/Th17 immune response that contribute to induction of colon tumorigenesis by ETBF. We will use two major approaches to address this question. A. We will identify the contribution of Stat3 activation in distinct cellular compartments to ETBF colon tumorigenesis. B. We will determine the contribution of specific Th17 cytokines in the induction of colon tumorigenesis by ETBF. 2) Analyze the effects of ETBF Th17 colitis on the genetic and epigenetic characteristics of induced colon tumors.
Role: Co-Investigator
Overlap: None

Award ID: R01CA142779 (Pardoll)
Title: B7-H1/PD1 modulation in cancer therapy
Effort: .96 calendar months
Supporting Agency: NIH/NCI
Name of Procuring Contracting/Grants Officer: Jason Gill
Address of Funding Agency: 9609 Medical Center Drive, Rockville, MD 20850
Performance Period: 06/21/2010 – 05/31/2015
Level of Funding: $266,585 annual direct cost
Project's Goal: The major goal of this project is to study B7-H1/PD1 modulation in cancer therapy.
Specific Aims: 1): Evaluate B7-H1 and PD-1 protein expression as independent markers of cancer progression. 2): Define mechanisms that regulate B7-H1 expression by tumors and PD-1 expression by T cells. 3): Dissect and manipulate B7-H1-mediated retrograde signaling in tumor cells ("B7-H1 molecular shield"). 4): Characterize immunological mechanisms underlying the clinical effects of B7-H1/PD-1 blockade in cancer therapy.
Role: PI
Overlap: None

Award ID: 90038966 (Pardoll)
Title: Combinatorial Immunotherapy for Melanoma with B7H1/PD-1 Checkpoint Blockade
Effort: 1.68 calendar months
Supporting Agency: Melanoma Research Alliance
Name of Procuring Contracting/Grants Officer: Laura Brockway-Lunardi, Ph.D.
Address of Funding Agency: 1101 New York Avenue, Suite 620, Washington, DC 20005
Performance Period: 7/15/09 – 08/31/14
Level of Funding: $500,000 annual direct cost
Project's Goal: The major goal of the project is to evaluate the combination of a GM-CSF transduced melanoma vaccine together with anti-PD1 blockade in the treatment of melanoma.
Specific Aim: N/A
Role: PI
Overlap: None

Award ID: U19AI088791 (Cox)
Title: Baltimore Acute Hepatitis C Cooperative Center
Effort: 0.36 calendar months

Supporting Agency: NIH/NIAID
Name of Procuring Contracting/Grants Officer: Samantha J. Tempchin
Address of Funding Agency: 6700B Rockledge Drive, Bethesda, MD 20817
Performance Period: 04/15/10 – 03/31/15
Level of Funding: $435,436 annual direct cost
Project's Goal: This grant is a multi-project grant permitting maintenance of a cohort of subjects at risk for HCV infection, studying humoral responses to HCV, and developing a unique set of in vitro and in vivo assays to assess the effects of immunomodulatory agents on HCV specific T cells.
Specific Aims: 1) To perform a set of in vitro functional analyses assessing the capacity of HCV specific CD8 T cells of various previously characterized phenotypes to produce relevant effector cytokines and to perform killer functions. This analysis will use blocking antibodies and agonists for selected inhibitory, survival and costimulatory receptors thought to play a potential role in regulating T cell responsiveness in the setting of chronic HCV infection. 2) To develop and characterize a novel in vivo cytotoxic lymphocyte (CTL) assay for functional analysis of HCV specific CD8 cells using adoptive transfer into NOD/SCID/-YC-/- (NOG) mice. This assay will allow us to directly analyze the in vivo functional effects of antibodies and/or cytokines targeted at potentially relevant cell membrane receptors on human HCV specific CD8 T cells. Using specific antagonist antibodies, candidate molecular determinants of CDS T cell unresponsiveness will be interrogated in this novel in vivo system in which human T cells from HCV infected patients are adoptively transferred into receptive immunodeficient mice. Outcomes of this interrogation will have direct translational relevance to the immunotherapy of chronic HCV infection as well as enhancing understanding of T cell impairment associated with persistent infection.
Role: Co-Investigator Project 1
Overlap: None

Award ID: 90053935 (Pardoll)
Title: Development of *STINGVAX*, Cyclic Dinucleotide Formulated GVAX Cancer Vaccine, as a Novel Cancer Immunotherapeutic Agent
Effort: 0.12 calendar months
Support Agency: Aduro Biotech
Name of Procuring Contracting/Grants Officer: Thomas Dubensky
Address of Funding Agency: 626 Bancroft Way, 3C, Berkeley, CA 94710-2224
Performance Period: 03/01/2013-02/28/2015
Level of Funding: $150,000 annual direct cost
Project's Goal: To provide critical preclinical studies to optimize STINGVAX and to define a treatment regimen for clinical use.
Specific Aims 1): To optimize the STINGVAX formulation with optimal *in vivo* tumor regression potential. 2): To establish the mechanism of action of STINGVAX in its ability to activate antigen presenting cell. 3): To assess the activation potential of the various CDN species on human dendritic cells
Role: PI
Overlap: None

Award ID: 90054405 (Sears)
Title: Induction of Human Colon Cancer by Bacteroides fragilis Toxin(BFT)-producing Bacteroides Species
Effort: 0.6 calendar months
Supporting Agency: Institut Merieux
Name of Procuring Contracting/Grants Officer: Christine M'Rini
Address of Funding Agency: 17 Rue Bourgelat-69002 Lyon (France)

Performance Period: 04/22/2013-04/22/2015
Level of Funding: $237,258 annual direct cost
Project's Goal: The major goal of this project is to validate the association of bft-expressing Bacteroides species with CRC and further, to identify targets for development of sensitive and specific diagnostics to identify patients at potential high risk for CRC
Specific Aim: N/A
Role: PI
Overlap: None

Award ID: 90056159 (Pardoll)
Title: Effects of mutation specific targeted TKI on tumor immunity and PD-1 ligand expression in NSCLC
Effort: .12 calendar months
Supporting Agency: Bristol-Myers Squibb Co
Name of Procuring Contracting/Grants Officer: Les Enterline
Address of Funding Agency: Route 206 and Providence Line Road, Princeton, NJ 08543
Period of Performance: 08/24/2013-08/23/2014
Level of Funding: $37,791 annual direct costs
Projects Goal: The goal of this project is to provide the basis to integrate driver oncogene-mutation directed TKI therapy with checkpoint PD-1 pathway blockade.
Specific Aims: Aim 1: In vitro analysis of effects of targeted TKI on T cell proliferation and cytokine production. Aim 2: Analysis of PD-1 ligand expression in pre- and post-targeted TKI treatment samples from NSCLC patients with defined mutations.
Role: PI
Overlap: None

Individuals who have worked on the project

The Regents of the University of California

Name:	Dennis Slamon, M.D., Ph.D.
Project Role:	PI (Senior/Key Personnel)
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Slamon contributes clinical, translational, and genomic expertise to the project and is involved in the overall direction.
Funding Support:	See Other Support

Name:	Judy Dering, Ph.D.
Project Role:	Sr Public Analyst
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Dering is responsible for analyzing data from the microarray experiments.
Funding Support:	No change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yes. See next pages for Dr. Slamon's Other Support.

OTHER SUPPORT

SLAMON, DENNIS

CURRENT

P30 CA016042 (PI: Gasson)
Title: "Cancer Support Grant"
Time Commitment: 1.80 calendar
Supporting Agency: NIH/NCI
Procuring Contracting/Grants Officer: Amy Connolly, Grant Management Specialist
Address of Grants Officer: National Cancer Institute, Room 700, Mail Stop 8335
6116 Executive Blvd, Bethesda, MD 20852-8335
Performance Period: 4/23/2003-11/30/2015
Level of Funding: $57,825
Project's Goal(s): This Funding supports activities to increase scientific interaction among members of the Signal Transduction Program Area at Jonsson Cancer Center.
Specific Aims:
Project Overlap or Parallel: No scientific or budgetary overlap.

W81XWH-11-1-0104 (PI: Slamon)
Title: "An Integrated Program in Translational Research in Human Breast Cancer"
Time Commitment: 2.40 calendar
Supporting Agency: DOD/ US Army
Procuring Contracting/Grants Officer:
Address of Grants Officer: Cheryl Lowery
820 Chandler Street, Fort Detrick, MD 21702-5014
Performance Period: 03/01/2011-03/31/2016
Level of Funding: $7,700,000
Project's Goal(s): Using newer approaches which include more modern and sophisticated genomic and proteomic technologies and a full panel of molecularly characterized human breast cancer cell lines representing the known subtypes of the diseases coupled with annotated tissue specimens, the team is seeking to address several major issues relevant to not only HER2-positive disease but the other subtypes comprising the breast cancer problem.
Specific Aims: Aim 1. Identify molecular alterations in human breast cancer cell lines that facilitate either inherent or treatment-acquired resistance of tumor cells to either lapatinib or trastuzumab. Aim 2. Retrospectively evaluate each molecular alteration, identified in aim 1, in breast cancer specimens using tissue microarrays prepared from breast cancers of women who were subjects in large clinical trials using HER2-targeted agents and determine if these alterations are associated with treatment resistance. Aim 3. Use of model systems to assess the potential role of identified molecular alterations in treatment resistance and evaluate pharmacological agents designed to inhibit the alteration(s). Aim 4. Design and implement clinical trials to assess the efficacy of available secondary agents that target factors associated with resistance in aims 1-3, in order to improve response rates to trastuzumab or lapatinib treatment.
Project Overlap or Parallel: No scientific or budgetary overlap.

A5481023 (PI: Slamon)
Tittle: Multicenter, Randomized, Double-Blind, Placebo-Controlled, Phase 3 Trial of Fulvestrant (Faslodex®) With or Without PD-0332991 (Palbociclib) ± Goserelin in Women with Hormone Receptor-Positive, HER2-Negative Metastatic Breast Cancer Whose Disease Progressed After Prior Endocrine Therapy.

Procuring Contracting/Grants Officer: Soo Y. Bang
Address of Grants Officer: Address of Contract officer: 235 E. 42nd Street, MS 685/13/1, New York, New York 10017
Performance Period: 11/26/13-11/26/17
Level of Funding: 345,811
Project's Goal(s): is to demonstrate the superiority of palbociclib in combination with fulvestrant (with or without goserelin) over fulvestrant alone (with or without goserelin) in prolonging investigator-assessed PFS in women with HR+/HER2-negative metastatic breast cancer whose disease has progressed on prior endocrine therapy.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

CIRM DR3-07067 (PI: Slamon)
Title: "A Phase I dose escalation and expansion clinical trial of the novel first-in-class Polo-like Kinase 4 (PLK4) inhibitor, CFI-400945 in patients with advanced solid tumors"
Time Commitment: 3.60 calendar
Supporting Agency: California Institute for Regenerative Medicine
Procuring Contracting/Grants Officer: Doug Kearney, Grants Management Office
Address of Grants Officer: California Institute for Regenerative Medicine, 210 King Street San Francisco, CA 94107
Performance Period: 03/01/2014-2/28/2017
Level of Funding: $8,469,697
Project's Goal(s): This proposal is aimed at a phase I clinical trial of CFI-400945, a first-in-class inhibitor of Polo-like Kinase 4 (PLK4). PLK4, a serine/threonine kinase functions at the intersection of mitosis, DNA repair, hypoxia and metabolism, and is expressed in a variety of solid tumors. Overexpression of PLK4 results in the excessive formation of centrioles and multinucleation in cells suggesting that the elevated expression of PLK4 in tumors could contribute to chromosomal instability (CIN) and aneuploidy. Of interest, PLK4 overexpression in neural stem cells drives centrosome amplification and is associated with tumor formation. Conversely, depletion of PLK4 in cancer cells by RNA interference prevents centriole duplication, causing mitotic defects and cell death. Notably, these effects are amplified in hypoxic conditions. Thus, PLK4 is an attractive target for the development of small-molecule therapeutics in cancer. The candidate molecule, CFI-400945 was developed as part of a collaborative effort funded by CIRM/CSCC (PIs: Dennis Slamon and Tak Mak) that supported a drug discovery effort, preclinical assessment, and IND enabling studies.
Specific Aims: This clinical trial described herein will be carried out in two parts. Part A will consist of the dose escalation phase of the first-in-human trial, where the primary objective will be to determine the maximum tolerated dose (MTD) of CFI-400945. In Part A, patients with any solid tumor refractory to conventional treatment will be enrolled in order to reach the MTD expeditiously. Part B will consist of the expansion phase, where the primary objectives are to further refine the MTD to assist in determination of the recommended phase II dose (RP2D), to further assess plasma pharmacokinetics and to evaluate preliminary evidence of antitumor activity patient populations dosed at the MTD. Up to 4 expansion cohorts of 6-12 patients each would be enrolled which may include: 1) cohorts restricted to a specific tumor histology and/or specific biomarker (predicated upon preclinical data) and a 2) a biomarker cohort to obtain tumor biopsy samples at pre-treatment, on-treatment, with the exploratory objective of evaluating pharmacodynamic effects and potential resistance mechanisms. We expect that the dose escalation will complete enrollment in approximately 1 year and an additional 12-18 months for completion of the expansion cohorts. We then expect an additional one year period will be required to collect data and complete a clinical study report (CSR). We believe that this Phase 1/1B trial will provide critical clinical and biomarker data that will demonstrate clinical proof of concept which will

inform the Phase 2 development plan. Over the next 4 years, our Phase I trial will also advance a successfully completed CIRM funded-project for which an IND has already been filed
Project Overlap or Parallel: No scientific or budgetary overlap.

W81XWH-14-1-0385 (PI: Baylin)
Title: A New Paradigm for the treatment of Ovarian Cancer: The use of Epigenetic Therapy to Sensitize Patients to Immunotherapy and Chemotherapy.
Time Commitment: 0.60 Calendar Months
Supporting Agency: US Army Subaward with John Hopkins University
Procuring Contracting/Grants Officer: Melody Snow, M.H.S, Assistant Director, Outgoing Awards
Address of Grants Officer: John Hopkins University, School of Medicine, 1629 Thames Street, Suite 200
Baltimore, Maryland 21231
Performance Period: 9/30/2014-9/29/19
Level of Funding: $314,280
Project's Goal(s): To robustly prolong the survival of patients with serous ovarian cancer (OC) through introducing epigenetic therapy paradigms.
Specific Aims: 1) To uncover the mechanisms through which epigenetic therapy may, alone, achieve robust, durable responses in patients with advanced ovarian cancer (OC) 2) Study how epigenetic therapy may sensitize OC cells to subsequent chemotherapies 3) Study how epigenetic therapy may sensitize OC cells to subsequent immunotherapies which targets checkpoints which are driving immune tolerance 4) Develop new combinations of epigenetic drugs which may provide for the highest level of efficacy for management of OC
5) Bring all of the above studies to bear on leveraging clinical trials of epigenetic therapy on OC
Project Overlap or Parallel: No scientific or budgetary overlap.

AWARDED SINCE LAST SUBMISSION

R01CA182514-01A1 (PI: Curtis)
Title: Intergrated genomic analysis and multi-scale modeling of therapeutic resistance
Time Commitment: 0.24
Supporting Agency: NIH Subaward with Stanford University
Procuring Contracting/Grants Officer: Aida Vasquez, Vasquez@mail.nih.gov
240-276-6319
Performance Period: 09/12/14-8/31/19
Level of Funding: $74,773
Project's Goal(s): The major goals of this project are to i) perform an integrated genomic analysis of serial tissue specimens from HER2-positive patients enrolled in clinical trials to evaluate the efficacy of single or dual agent neoadjuvant lapatinib and or trastuzumab targeted therapy (NCT00769470/TRIO B07) in order to characterize mechanisms of resistance ii) delineate temporal patterns of clonal expansions under treatment selective pressure by analyzing longitudinal samples collected prior to, at run-in, and after therapy iii) to functionally characterize mechanisms of resistance to single and dual agent therapy in HER2-positive tumors and to phenotype resistant cell populations by analyzing patient-derived xenograft models and short-term primary cultures.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

COMPLETED SINCE LAST SUBMISSION

Stand Up to Cancer Dream Team (PI: Slamon)
Title: "An integrated Approach to Targeting Molecular Breast Cancer Subtypes and Their Resistance Phenotypes"
Time Commitment: 1.20 calendar
Supporting Agency: American Association for Cancer Research
Procuring Contracting/Grants Officer: Michael Stewart, CFO
Address of Grants Officer: American Association for Cancer Research, 615 Chestnut Street, 17th FL
Philadelphia, PA 19106-4404
Performance Period: 10/01/2009-09/30/2014
Level of Funding: $15,000,000
Project's Goal(s): The goals and objectives of this "Stand Up to Cancer" (SU2C)/AACR Breast Cancer Dream Team is to undertake a fully-integrated, molecular, genomic, biologic and "informatics" translational research approach directed at development of new and more effective therapies for the spectrum of diseases that comprise human breast cancer.
Specific Aims: ***Specific Aim I*** – Expand our understanding of the known "driving" initial molecular mechanisms responsible for the pathogenesis and clinical behavior of the three known therapeutic breast cancer subtypes, i.e. estrogen (E2)/estrogen receptor (ER-positive), HER2-positive and triple-negative (TN) subtypes of breast cancers. This will be accomplished using existing and/or creating new, relevant preclinical models as well as querying annotated clinical materials with the latest technologies and informatics platforms. The ultimate objective of this effort will be the design, development and clinical testing of new and innovative therapies for the known molecular subtypes of breast cancer;
Specific Aim II - Study the "driving" mechanisms responsible for *de novo* as well as acquired resistance to appropriately targeted treatments of the three known therapeutic breast cancer subtypes, i.e. estrogen (E2)/estrogen receptor (ER-positive), HER2-positive and triple-negative (TN) breast cancers. As in Specific Aim I, this will be accomplished utilizing existing and/or creating new, relevant preclinical models of resistance to current therapeutics as well as querying annotated clinical materials exhibiting the "resistance" phenotype using the latest technologies and informatics platforms. Again, the ultimate objective will be the design, development and clinical testing of new and innovative therapies for the "resistance" phenotype in the known breast cancer subtypes;
Specific Aim III - Investigate the potential initial "driving" pathogenetic as well as *de novo* or acquired "resistance" mechanisms mediated by "stem/progenitor" breast cancer cells within each or across all of the three known breast cancer therapeutic subtypes with the ultimate objective being the design, development and clinical testing of new and innovative therapies for the "tumorigenic" and "resistance" phenotypes potentially mediated by these stem/progenitor cells;
Specific Aim IV - Develop new and/or characterize existing relevant and representative cell line and xenograft models as well as utilize annotated clinical material to query the contributions of "normal" and "malignancy-derived" matrix/stromal components of each breast cancer subtype including those that might contribute to or mediate the "resistance" phenotype to targeted therapeutics. The ultimate objective will again be the design, development and clinical testing of new and innovative therapies for the "tumorigenic" and "resistance" phenotypes potentially mediated by these matrix/stromal components;
Specific Aim V - Develop an integrated discovery and informatics research unit that cuts across the above Specific Aims that is designed to deploy, inform and facilitate implementation of relevant discovery and informatics platforms needed for these aims. This will include utilization of robust informatics and systems biology efforts to not only manage, integrate and disseminate relevant data between Dream-Team members and the greater scientific community, but also to execute genome-

wide analyses of relevant genes/pathways and "nodes" critical to breast cancer subtype pathogenesis and directed at identification of "resistance" mechanisms. This effort will also lead to further refinement of the current molecular classification of known breast cancer therapeutic subtypes. As with all other Specific Aims, this Aim will have as its ultimate objective, the design, development and clinical testing of novel and hopefully more effective, less toxic therapies for women challenged with breast cancer both in the adjuvant and metastatic settings.
Project Overlap or Parallel: No scientific or budgetary overlap.

DR1-01477 (PI: Slamon)
Title: "Therapeutic Opportunities to Target Tumor Initiating Cells in Solid Tumors"
Time Commitment: 3.60 calendar
Supporting Agency: CIRM (Disease Team Awards)
Procuring Contracting/Grants Officer: Doug Kearney, Grants Management Office
Address of Grants Officer: California Institute for Regenerative Medicine, 210 King Street San Francisco, CA 94107
Performance Period: 05/01/2010-04/30/2014
Level of Funding: $19,979,660
Project's Goal(s): The purpose of the California Institute for Regenerative Medicine (CIRM) Disease Team Research Awards is to accelerate potential therapies based on stem cell research toward clinical testing. To facilitate this goal, CIRM intends to support actively managed multidisciplinary teams engaged in milestone-driven translational research. We propose to develop novel therapeutic drugs that target solid tumors affecting the brain, colon and ovaries.
Specific Aims: (1) increase the number of characterized xenografts and CIC-enriched cell lines, (2) carry out genomic characterization of these xenografts and cell lines, (3) carry out genomic characterization of available tumor bank samples, (4) test candidate PLK4 and TTK inhibitors supplied by the drug discovery group, (5) carry out combination drug testing, and (6) analyze the data en masse to determine how various CIC subtypes and tumor samples respond to the drugs so that optimal compounds can be selected and a targeted clinical plan developed.
Project Overlap or Parallel: No scientific or budgetary overlap.

OAM4861g (PI: Slamon)
Title: "A Randomized, Phase II, MultiCenter, Double-Blind, Placebo-Controlled study evaluating the safety and efficacy of Metmab and/or Bevacizumab in combination with Paclitaxel in patients with metastatic triple negative breast cancer"
Time Commitment: 0.12 calendar
Supporting Agency: Genentech, Inc
Procuring Contracting/Grants Officer: See-Chun Phan, M.D.
Address of Grants Officer: 1 DNA way, South San Francisco, CA 94080-4990
Performance Period: 12/01/11-02/28/15
Level of Funding: $192,211
Project's Goal(s): The goals of the OAM4861g study are to estimate the clinical benefit of MetMAb + bevacizumab + paclitaxel and MetMab + Placebo + Paclitaxel Relative to Placebo + bevacizumab + paclitaxel.
Specific Aims: To characterize the safety and tolerability of MetMAb + bevacizumab + paclitaxel and MetMAb + placebo + paclitaxel relative to placebo + bevacizumab + paclitaxel, To evaluate drug exposure of MetMAb, paclitaxel, and bevacizumab, To evaluate the serum levels and incidence of anti-therapeutic antibodies (ATAs) against MetMAb,To evaluate the effect of MetMAb on the following electrocardiogram (ECG) parameters: corrected QT (QTc) interval, heart rate, uncorrected QT interval, PR interval, QRS duration, and T-wave and U-wave morphology.
Project Overlap or Parallel: No scientific or budgetary overlap.

BCIRG#006 (PI: Slamon)
Title: "MC, PH III R, Trial Comp (AC-T), (AC-TH), oor (TCH) in the TX of NODE = & high risk node – adjuvant w Operable breast cancer containing ain HER2NEU alter"
Time Commitment: 0.12 calendar
Supporting Agency: Breast Cancer International Research Group
Procuring Contracting/Grants Officer: Ira Steinberg, M.D.
Address of Grants Officer: 55 Cambridge Parkway, Cambridge, Massachusetts 02142
Performance Period: 06/01/01-12/31/14
Level of Funding: $2,177,946
Project's Goal(s): The goals of the BCIRG #006 study is to compare disease free survival after treatment with doxorubicin and cyclophasphamide followed by docetaxel with doxorubicin and cyclophosphamide followed by docetaxel and trastuzumab and with docetaxel in combination with platinum salt and herceptin in the treatment of node positive and high risk node negative adjuvant patients.
Specific Aims: Compare overall survival between above mentioned arms. Compare toxicity and quality of life between mentioned arms, evaluate pathologic and molecular markers for predicting efficacy**.**
Project Overlap or Parallel: No scientific or budgetary overlap.

BCIRG#005 (PI: Slamon)
Title: "MC/P3/R Trial comparing Docetaxel in COMB w/ Doxorubicon & Cyclophosphamide followed by Docetaxel"
Time Commitment: 0.10 calendar
Supporting Agency: Breast Cancer International Research Group
Procuring Contracting/Grants Officer: Dominique Mery-Mignard, Medical Director
Address of Grants Officer: 42-50 Qua de la rapee, 75012 Paris, France
Performance Period: 06/01/2001-12//31/2013
Level of Funding: $1,066,299
Project's Goal(s): The Goals of the BCIRG#005 study is to compare disease free survival after treatment with Docetaxel in combination with doxorubicin and cyclophosphamide (TAC) in operable breast cancer HER2neu negative patients with positive axillary lymph nodes.
Specific Aims: Compare overall survival between above mentioned arms. Compare toxicity and quality of life between mentioned arms, evaluate pathologic and molecular markers for predicting efficacy**.**
Project Overlap or Parallel: No scientific or budgetary overlap.

Individuals who have worked on the project

Van Andel Research Institute

Name:	Peter Jones, Ph.D.
Project Role:	PI (Senior/Key Personnel)
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Jones serves as PI on this project.
Funding Support:	See Other Support

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yes. See next pages for Dr. Jones' Other Support.

OTHER SUPPORT

JONES, PETER A.

Current Support

R01 CA 082422 (PI: Jones) Previously R37

Title:	Mechanisms of *De Novo* Methylation in Cancer
Time Commitment:	3.0 calendar
Supporting Agency:	NIH/NCI
Procuring Contracting/Grants Officer:	Sy Shakleford
Address of Grants Officer:	National Cancer Institute, Executive Plaza North, Suite 5024, 6130 Executive Blvd., Rockville, MD 20852
Performance Period:	09/17/99-7/31/19
Level of Funding:	$290,706 Annual Direct
Project's Goals:	The major goals of this project are to study mechanisms of *de novo* methylation in culture systems and to probe the potential roles of chromatin structure in defining DNA methylation patterns.
Specific Aims:	1) To determine the role of DNA methylation on the structure of the cancer epigenome and to elucidate the potential mechanisms by which histone methyltransferases and chromatin alter the stability and output of the human epigenome; 2) determine the immediate epigenome-wide outcomes of treating cells with the global DNA methylation inhibitor 5-aza-2'-deoxycytidine and then probe the kinetics of remethylation and its link to chromatin remodeling with a particular focus on gene body methylation; 3) to complete the first pilot epigenome maps of a small number of uncultured human colon tumors and compare them to adjacent tissue collected from same patients.
Project Overlap or Parallel:	No scientific or budgetary overlap.

5 R01 CA 083867 (PI: Jones/Liang)

Title:	*De Novo* DNA Methylation in Bladder Cancer
Time Commitment:	1.80 calendar
Supporting Agency:	NIH/NCI
Procuring Contracting/Grants Officer:	Paul Okano
Address of Grants Officer:	National Cancer Institute, Executive Plaza North, Suite 5024, 6130 Executive Blvd., Rockville, MD 20852
Performance Period:	03/01/2000-04/30/2016
Level of Funding:	No Cost Extension
Project's Goals:	The goal of this project, which has been funded for almost 30 years, is to understand the genetic and epigenetic basis of human bladder cancer.
Specific Aims:	1) Use a series of eight hypermethylation markers to complete the examination of DNA in urine sediments obtained from individuals with low grade tumors to determine whether we can detect the frequent recurrences of these tumors; 2) complete the analysis of DNA from healthy individuals of different ages to

	determine whether age-related changes in DNA methylation can be detected in urine sediments; 3) determine the functional significance of an observed hypomethylation phenotype by analyzing directly whether methylation of non- CpG island regions which constitute the bulk of the phenotype might be involved in gene activation and have chromatin properties associated with active genes; 5) take advantage of ongoing clinical trials in which patients with myelodysplastic syndrome are being treated with the hypomethylating drug 5-azacytidine (5-aza-CR). This grant does not overlap with the proposed research as it does not investigate the role of ascorbic acid in cancer treatment.
Project Overlap or Parallel:	No scientific or budgetary overlap.

1 R01 CA170550 (PI: Laird)

Title:	Epigenetic Drivers of Cancer (PQ 10)
Time Commitment:	1.20 calendar
Supporting Agency:	NIH/NCI
Procuring Contracting/Grants Officer:	Roy W. Tarnuzzer
Address of Grants Officer:	National Cancer Institute, 31 Center Drive, BG 31 Room 3A20, Bethesda MD 20814
Performance Period:	09/01/2012-06/30/2016
Level of Funding:	$589,952
Project's Goals:	The major goals of this project are addresses an unmet need to develop methods of finding out which epigenetic changes contribute directly to cancer formation.
Specific Aims:	1. We will develop a probabilistic framework for predicting and prioritizing candidate epigenetic driver loci. This approach is unique in that it fully integrates the wealth of available data, using complementary data types derived from primary genomic data, experimental data, and supporting curated information, resulting in a composite Epigenetic Driver Score (EDS), reflecting the posterior probability that each gene is an epigenetic driver. 2. Will provide experimental data on epigenetic addiction, using cell lines depleted of DNA methyltransferases, and thus selected to retain only the most essential silencing events, in addition to data obtained with embryonic and adult stem-cell and progenitors. These experimental data sets will be used to complement primary epigenomic data we have generated in the context of TCGA, to provide Epigenetic Driver Scores for each locus in each tumor type, using the methodology developed in Aim 1. 3a. We will functionally test the top-ranked candidate epigenetic drivers of colon, breast, and lung cancer in vitro, by reintroducing expression of candidate genes into appropriate human cancer cells lines containing the relevant silencing events. These experiments will be complemented by shRNA approaches in cell lines to modulate the functional expression of the candidate epigenetic drivers. *In vitro* proliferation and apoptosis assays will be used to assess phenotypic effects. 3b. We will assess the functional contributions of the candidate epigenetic drivers *in*

vivo, using the stable cell lines created in Aim 3a in xenograft mouse models.

Project Overlap or Parallel: No scientific or budgetary overlap.

W81XWH14-1-0385 (PI: Baylin/Jones)

Title: A New Paradigm for the Treatment of Ovarian Cancer: The Use of Epigenetic Therapy to Sensitize Patients to Immunotherapy and Chemotherapy

Time Commitment: 0.3 calendar

Supporting Agency: DoD/Department of the Army via Johns Hopkins University

Procuring/Contracting/Grants Officer: Barbara Schneider, Johns Hopkins University,

Address of Grants Officer: The Sidney Kimmel Comp Cancer Ctr, 1650 Orleans St., CRBI Rm352, Baltimore, MD, 21287-0013, schneba@jhmi.edu

Performance Period: 9/30/14 – 9/30/19

Level of Funding: $38,591 Annual Direct

Project Goals: The goal of this project is to determine how DNMTs activate drug response pathways in ovarian cancer.

Specific Aims: 1) To uncover the mechanisms through which epigenetic therapy may, alone, achieve robust, durable responses in patients with advanced ovarian cancer (OC); 2) Study how epigenetic therapy may sensitize OC cells to subsequent chemotherapies; 3) Study how epigenetic therapy may sensitize OC cells to subsequent immunotherapies which targets checkpoints which are driving immune tolerance; 4) Develop new combinations of epigenetic drugs which may provide for the highest level of efficacy for management of OC; 5) Bring all of the above studies to bear on leveraging clinical trials of epigenetic therapy on OC

Project Overlap or Parallel: No scientific or budgetary overlap.

<u>Previous Support</u>

5 P30 CA 014089 (PI: Jones)

Title: USC Norris Comprehensive Cancer Center (Core) Grant

Time Commitment: 6.0 calendar

Supporting Agency: NIH/NCI

Procuring Contracting/Grants Officer: Connie Murphy

Address of Grants Officer: Department of Health and Human Services, National Institutes Of Health, National Cancer Institute

Performance Period: 12/01/05-11/30/11

Level of Funding: $4,254,556 Total

Specific Aims: N/A

Project's Goals: This grant supports 50% of my salary in my role as Director of the USC Norris Comprehensive Cancer Center.

Project Overlap or Parallel: No scientific or budgetary overlap.

Stand Up to Cancer (PIs: Baylin/Jones)

Title: Bringing Epigenetic Therapy to the Forefront of Cancer Management

Time Commitment: 1.08 calendar

Supporting Agency: American Association for Cancer Research

Procuring Contracting/Grants Officer: Alexandra Sedehi, J.D.
Address of Grants Officer: The Johns Hopkins University – SOM
Broadway Research building
733 North Broadway, Suite 117
Baltimore, MD 20205
Performance Period: 12/01/2009-11/30/2013
Level of Funding: $752,952 (NCE)
Project's Goals: The major goal of our multi-institutional Dream Team consists of experts in the epigenetics of cancer aiming to bring, in 3 years, epigenetic therapy to the forefront of cancer management.
Specific Aims: 1. To develop molecular markers which predict, and monitor, the efficacy of cancer epigenetic therapies. 2. To perform clinical trials to bring epigenetic therapy to the forefront of cancer management. 3. To determine whether a key mechanism for efficacy of epigenetic therapy is targeting and exhaustion of self-renewing cancer cells. 4. To develop a clinical trial with a new drug designed to circumvent the instability of 5-AC and DAC. 5. To determine targets in addition to promoter DNA hypermethylation that may be utilized in new cancer epigenetic therapy approaches.
Project Overlap or Parallel: No scientific or budgetary overlap.

5 T32 CA 009320 (PI: Jones)
Title: Training Grant in Viral and Chemical Carcinogenesis
Time Commitment: 0.60 calendar
Supporting Agency: NIH/NCI
Procuring Contracting/Grants Officer: Susan E. Lim
Address of Grants Officer: National Cancer Institute, 6116 Executive Boulevard, Room 7043 Rockville, MD 20852
Performance Period: 08/01/1979-06/30/2014
Level of Funding: $284,136
Project's Goals: The major goal of this project is to support five postdoctoral fellows for two years each at the USC Norris Cancer Center. I was listed as a PI with 5% effort but no salary support.
Specific Aims: 1. Trainees will conduct research under the supervision of a member of the Norris faculty. 2. Trainees will attend Grand Rounds, a seminar series that brings together clinicians and basic scientists on topics of mutual interest; third, trainees will present their work at one or more discipline-based research seminars, including eukaryotic gene regulation, epigenetics, and developmental biology. 3. Trainees will have the option of attending didactic courses in a variety of subjects related to cancer biology, including courses in molecular genetics, human genetics, developmental biology, pathology, cancer biology, responsible conduct in research (required), and computational biology.
Project Overlap or Parallel: No scientific or budgetary overlap.

5 R01 CA 138794 (PI: Liang)
Title: Determining the Mechanistic and Therapeutic Roles of microRNAs in Bladder Cancer

Time Commitment: 0.60 calendar
Supporting Agency: NIH/NCI
Procuring Contracting/Grants Officer: Tawnya C. McKee
Address of Grants Officer: National Cancer Institute, Molecular Targets Development Program, Center for Cancer Research, Frederick, Maryland
Performance Period: 08/09/2010-06/30/2014
Level of Funding: $260,852
Project's Goals: The major goal of this project is to provide an exciting step towards the clinical application of using miRNAs as diagnostic and/or prognostic markers and as therapeutic targets in bladder cancer patients.
Specific Aims: 1. Identifying specific miRNAs for diagnostic and prognostic purposes for bladder cancer patients. 2. Reactivating silenced tumor suppressor miRNAs by epigenetic treatment. 3. Characterizing the role of miRNAs during tumorigenesis and re-expressing identified tumor suppressor miRNAs in cancer cells with a multiple miRNA expression vector.
Project Overlap or Parallel: No scientific or budgetary overlap.

5 U24 CA 143882 (PI: Laird)
Title: The USC-JHU Cancer Epigenome Characterization Center
Time Commitment: 0.60 calendar
Supporting Agency: NIH/NCI
Procuring Contracting/Grants Officer: Roy W. Tarnuzzer
Address of Grants Officer: National Cancer Institute, 31 Center Drive, BG 31 Room 3A20, Bethesda MD 20814
Performance Period: 09/29/2009-07/31/2014
Level of Funding: $2,062,357
Project's Goals: The major goal of this project is to participate in the TCGA as the major epigenetic mapping center.
Specific Aims: 1. To characterize the DNA methylation status of 27,578 CpG dinucleotides located in 14,495 gene promoters in at least 10,000 human cancer samples and 1,000 control samples using the lllumina Infinium DNA Methylation analysis platform. 2. To transition epigenomic data production in TCGA to whole genome shotgun bisulfite sequence analysis to provide single-base-pair resolution DNA methylation data for TCGA cancer samples**.** 3. To implement quality control and quality assurance measures to ensure that epigenomic data deposited for public dissemination meets rigorous standards
Project Overlap or Parallel: No scientific or budgetary overlap.

2002195337 (PI: Baylin)
Title: The Intersection of Epigenetic and Immune Checkpoint Therapy
Time Commitment: 1.2 calendar
Supporting Agency: American Association for Cancer Research via Johns Hopkins University
Procuring Contracting/Grants Officer: Barbara Schneider
Address of Grants Officer: Johns Hopkins University, The Sidney Kimmel Comp Cancer Ctr, 1650 Orleans St., CRBI Rm352, Baltimore, MD, 21287-0013, schneba@jhmi.edu

Performance Period:	7/1/14 – 6/30/15
Level of Funding:	$15,000
Project Goals:	The goal of this project is to determine the evidence for the intersection of epigenetic and immune checkpoint therapy.
Specific Aims:	1) Within the framework of clinical samples obtained during the course of the clinical trials currently being conducted to evaluate both epigenetic therapy with DNA demethylating agent and HDACi and anti-PD1 and anti-CTLA4; 2) For Adoptive cellular therapy, there is the opportunity to evaluate in a defined population of antigen specific T cells epigenetic changes following adoptive transfer, and, during the process of generating T cells for adoptive therapy, the opportunity to determine if epigenetic manipulation can favorably modulate the replicative capacity, effector function and/or differentiation phenotype of the final cell product; 3) Utilize results from all of the above studies to help craft leveraged clinical trials for lung, melanoma and other cancers which are based on hypotheses derived from the data.
Project Overlap or Parallel:	No scientific or budgetary overlap.

8. SPECIAL REPORTING REQUIREMENTS

Progress of the Teal Junior Scientist, Dr. Kate Chiappinelli

Scientific training: Dr. Chiappinelli has especially benefitted from working with collaborators in Germany, Drs. Reiner Strick and Pamela Strissel. The latter spent a month in the Baylin lab on sabbatical, and mentored Kate for learning how to profile, knockdown, and overexpress several of the endogenous retroviruses (ERVs). Dr. Strissel is an expert on the ERVs and a co-first author on the paper with Kate as a first author which appeared in Cell in August, 2015 (Figs. 1-4, sections above). Kate has also worked extensively with Dr. Cindy Zahnow's group to perform the experiments with immunocompetent mice which are discussed in detail in previous sections Figs. 5-10. A most significant part of her training has been learning how to isolate and profile mouse immune cells, both from the spleen and from tumor. Dr. Drew Pardoll's laboratory and the FACS Core at the Sydney Kimmel Cancer Center have also helped Kate significantly in this work which is also detailed earlier above as shown in Figs.5, 6, and 10.

Participation in Hopkins groups:

1) Methylation Data Group: attended these weekly meetings, and presented twice.
2) Methylation Journal Club: attended these weekly meetings, and presented twice.
3) Tumor Biology Lab Meeting: attended these weekly meetings, and presented once.
4) The Pan-Cancer Data Working Group: attended these monthly meetings, and presented once. Our Pan-Cancer paper is now published (Li, Chiappinelli *et al.,* Oncotarget 2014) and we have ended these meetings.
5) The Epigenetics and Vaccine Meeting: attended these bi-weekly meetings, and presented once.
6) The Ovarian Cancer Working Group: attended these monthly meetings and will present in June.
7) Shih/Wang Laboratory Meeting: attended once a month.
8) The Gynecological Oncology Tumor Board: attended once a month.
9) Pardoll Laboratory Meeting: attended once a month and Kate is on the thesis committee for Dr. Pardoll's graduate student, Brian Francica.
10) Met with co-investigator Peter Jones and his group at the Van Andel Research Institute in Grand Rapids, Michigan in July, 2015 and this group will come to Baltimore to meet in Nov. 2015.

National meetings:

Kate attended the AACR Annual Meeting in Philadelphia, PA (April 2015), receiving an AACR NextGenStar Award (one of 8) and was given the opportunity to present her work (15 minute oral presentation) in a Major Symposium.

International meetings:

1. Kate was chosen as a Travelling Fellow (one of 6) to attend the Helene Harris Memorial Trust 13th International Forum on Ovarian Cancer in Toledo, Spain (January 2015) where she gave a 15 minute oral presentation.
2. Oral presentation and poster at the Cancer Genetics and Epigenetics Gordon Conference in Lucca, Italy (April 2015).

Professional development:

1. Kate has been attending a series of lunch seminars in Fall 2015 run by the Johns Hopkins Postdoctoral Association (JHPDA) focused on "Your Research Career". Topics included "Faculty Job Search", "Project Management in Research," "Effective Mentoring", *etc.*
2. Attended workshops sponsored by the Preparing Future Faculty Teaching Academy (PFFTA) on teaching at the undergraduate and graduate levels.

3. Gave a guest lecture for molecular biology graduate students in *Biology 630: Mechanisms of gene regulation* taught by Dr. Raymond Enke at James Madison University.

Additional training:
1. Thesis committee member for Brian Francica, a Ph. D. student in Dr. Drew Pardoll's laboratory.
2. Co-mentor with Dr. Baylin for a third year Ph. D. student, Michael Topper.
3. Mentored Benjamin Akman, an undergraduate, during summer 2014. He is an author on the *Cell* manuscript.

Individual Development Plan (IDP) for Postdoctoral Fellows:
Johns Hopkins University School of Medicine requires postdoctoral fellows and their mentors to fill out an annual IDP. This allows the fellow and mentor to identify long-term and short-term goals for the postdoc's research progress as well as career development. Kate has completed an IDP and this is reviewed annually with Dr. Baylin.

Teal Innovator's Ovarian Cancer ambassadorship activities

Dr. Baylin has been very active this past year in discussing the exciting results which have evolved to date and which are outlined in the Progress Report. He has given plenary section lectures at multiple events including, among others, the national AACR meeting, the Cancer Genetics and Epigenetics Gordon Conference in Lucca, Italy (April 2015), the AACR conference on Epigenetics and Cancer in Atlanta, September, 2015, the annual American Society of Investigative Pathology meeting in Baltimore, Oct. 2015, Symposium, National Center for Protein Sciences, Oct. 20, Symposium, Salk Institute, Sept, 2015, Wilson Symposium, MD Anderson, Oct. 2015.

9. APPENDICES

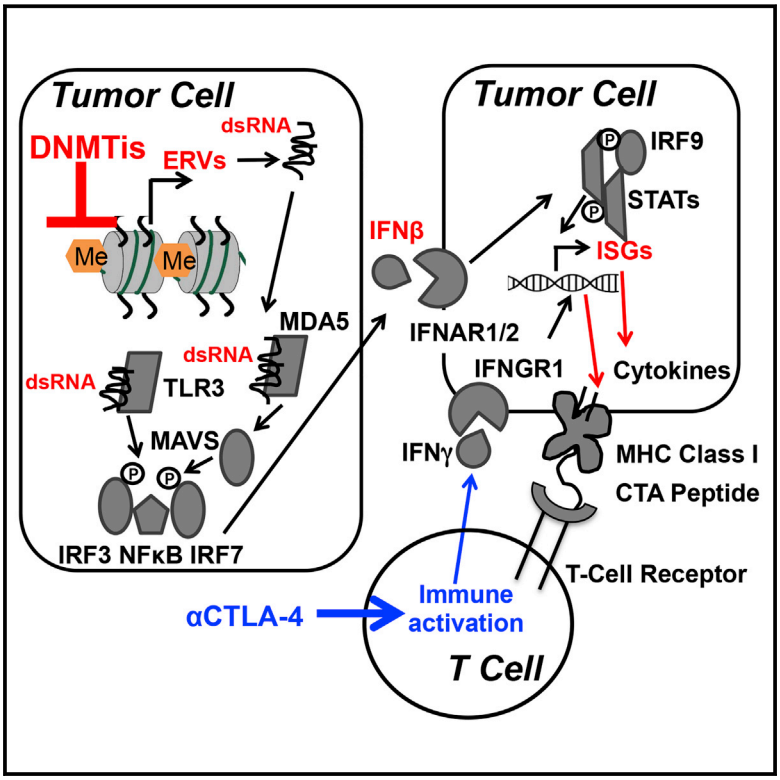
Chiappinelli KB, Strissel PL, Desrichard A, Li H, Henke C, Akman B, Hein A, Rote NS, Cope LM, Snyder A, Makarov V, Buhu S, Slamon DJ, Wolchok JD, Pardoll DM, Beckmann MW, Zahnow CA, Mergoub T, Chan TA, Baylin SB and Strick R. Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses. Cell. 162(5):974-86, 2015.

Article

Cell

Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses

Graphical Abstract



Highlights

- DNMTis induce an interferon response in cancer cells by activating dsRNA sensors
- DNMTis induce ERV demethylation and expression helping trigger the dsRNA response
- DNMTi viral defense genes in melanoma track with patient response to immune therapy
- DNMTi treatment sensitizes to anti-CTLA-4 immunotherapy in a melanoma mouse model

Authors

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In Brief

DNA methyltransferase inhibitors upregulate endogenous retroviruses in tumor cells to induce an growth-inhibiting immune response. High expression of the genes associated with the anti-viral response seems to potentiate a response to immune checkpoint therapy.

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CellPress

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Article

Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses

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SUMMARY

We show that DNA methyltransferase inhibitors (DNMTis) upregulate immune signaling in cancer through the viral defense pathway. In ovarian cancer (OC), DNMTis trigger cytosolic sensing of double-stranded RNA (dsRNA) causing a type I interferon response and apoptosis. Knocking down dsRNA sensors TLR3 and MAVS reduces this response 2-fold and blocking interferon beta or its receptor abrogates it. Upregulation of hypermethylated endogenous retrovirus (ERV) genes accompanies the response and ERV overexpression activates the response. Basal levels of ERV and viral defense gene expression significantly correlate in primary OC and the latter signature separates primary samples for multiple tumor types from The Cancer Genome Atlas into low versus high expression groups. In melanoma patients treated with an immune checkpoint therapy, high viral defense signature expression in tumors significantly associates with durable clinical response and DNMTi treatment sensitizes to anti-CTLA4 therapy in a pre-clinical melanoma model.

INTRODUCTION

DNA methyltransferase inhibitors (DNMTis) such as 5-azacytidine (Aza) and 5-aza-2′-deoxycytidine (Dac) are effective cancer therapies in hematologic neoplasms (Issa, 2005; Matei et al., 2012) and are Food and Drug Administration (FDA)-approved for the pre-leukemic disorder myelodysplasia (MDS) (Kaminskas et al., 2005). These cytidine analogs incorporate into DNA, block catalytic actions of DNA methyltransferases (DNMTs), and trigger their degradation (Stresemann et al., 2006). Preclinically, low doses avoid early cytotoxicity and DNA damage, allowing cells to exhibit apparent reprogramming and blunting of tumorigenicity (Tsai et al., 2012). Mechanisms can include reversal of abnormal promoter DNA methylation, re-expression of silenced genes including tumor suppressors (Baylin and Jones, 2011), and changes to cancer signaling pathways including apoptosis, cell-cycle activity, and stem cell functions (Tsai et al., 2012).

A long recognized activity of DNMTis described by others (Karpf et al., 1999, 2004) and our group (Li et al., 2014; Wrangle et al., 2013), is induction of immune responses in cancer cells. In recent clinical trials for non-small cell lung cancer (NSCLC) (Juergens et al., 2011; Wrangle et al., 2013) a small number of patients had remarkably robust and durable responses to immune checkpoint blockade therapy after first receiving Aza (Wrangle et al., 2013). This immune therapy alone also has activity against NSCLC (Brahmer et al., 2010, 2012; Topalian et al., 2012). A larger trial is now ongoing to determine if Aza can indeed prime patients for sensitization to checkpoint inhibition (Brahmer, 2015). For NSCLC and other tumor types, Aza induces interferon signaling and concordant upregulation of surface antigens and their assembly proteins, viral defense pathways, and transcript and surface protein levels of PD-L1, the key checkpoint ligand targeted in the above immunotherapy (Li et al., 2014; Wrangle et al., 2013). Indeed, we have defined a 300-gene expression signature we termed Aza-induced immune genes or AIM (Li et al., 2014) for which activation is greatest for epithelial ovarian cancer (EOC) and NSCLC (Li et al., 2014). Genome-wide expression of AIM separates primary EOC, NSCLC, and other cancers into high and low expression groups (Li et al., 2014). We hypothesize the low group may represent an ''immune evasion/immune editing'' pattern (Drake et al., 2006; Schreiber et al., 2011) that Aza could reverse to sensitize patients to subsequent immune therapy (Li et al., 2014).

We now show that a major mechanism underlying the Aza-triggered immune response is induction of a cytosolic

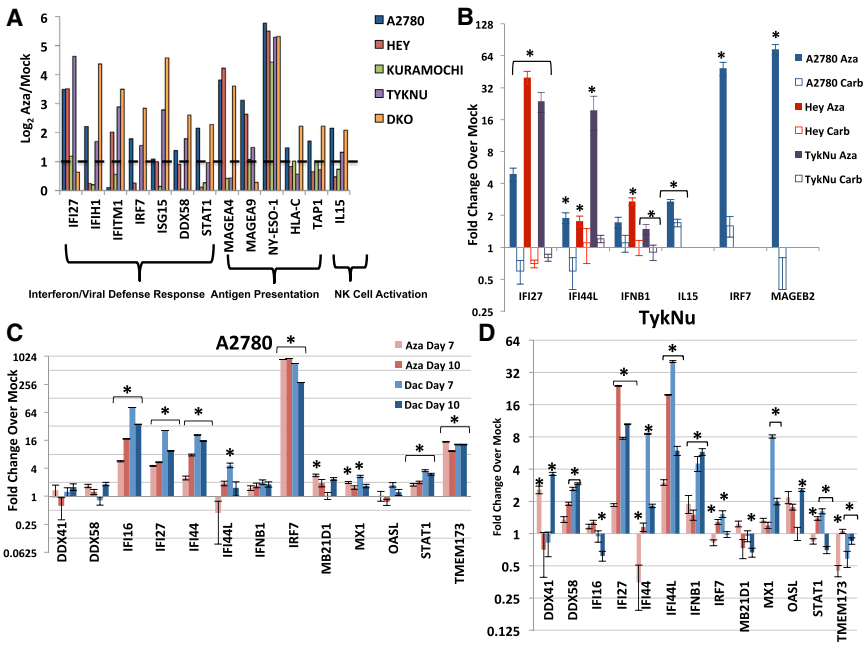

Figure 1. DNMT Inhibitors Upregulate Immune Genes in Ovarian Cancer Cell Lines

(A) Levels of immune genes in four EOC cell lines and DKO colon cancer cell line (DNMT1$^{-/-}$, DNMT3B$^{-/-}$) relative to its parental HCT116 line. y axis, $\log_2$ Aza/Mock fold change from microarray data. Dotted line denotes 2-fold change.

(B) qRT-PCR validation of immune genes in EOC cells treated for 72 hr: Mock, 500 nM Aza, or 500 nM- 3 μM carboplatin and rested for 7 days before assaying (day 10). IC_{50}s were A2780 (Aza, 848 nM; Carb, 457 nM), Hey (Aza, 4.1 μM; Carb, 12.2 μM), TykNu (Aza, 491 nM; Carb, 986.2 nM).

(C and D) qRT-PCR validation of interferon response genes in the A2780 (C) and TykNu (D) EOC lines treated with no drug (Mock), 500 nM Aza (Aza), or 100 nM Decitabine (Dac) for 3 days, and rested for 4 (day 7) or 7 (day 10) days before assaying. Data in (B)–(D) are represented as mean ± SEM of three biological replicates, y axis = fold change over mock. *p ≤ 0.05.

See also Figure S1.

double-stranded RNA (dsRNA) sensing pathway used by epithelial and other cell types as a viral defense mechanism that triggers a type I interferon response (Kulaeva et al., 2003; Sistigu et al., 2014). A key contributor is induction of increased expression of multiple DNA hypermethylated endogenous retroviruses (ERVs). In The Cancer Genome Atlas (TCGA), the viral defense gene expression separates primary EOC and other cancers into high and low expression and high tumor expression strongly associates with clinical benefit in a trial of immune checkpoint therapy for advanced melanoma. Aza sensitizes to immune checkpoint blockade in a pre-clinical model of melanoma. We thus define a potential approach in which an epigenetic therapy may sensitize cancer cells to various immunotherapies.

RESULTS

DNMTis Trigger Viral Defense and Type I Interferon Signaling

Induction of AIM in a previous study of 23 EOC cell lines (Li et al., 2014) included, in addition to previously reported DNA hypermethylated cancer testis antigens (*MAGEA4, MAGEA9, NY-ESO-1*) (James et al., 2013; Karpf et al., 2004, 2009; Odunsi et al., 2014), interferon/viral defense, antigen processing and presentation, and host immune cell attraction genes (Figure 1A). Direct Aza targeting of DNMTs for these changes is suggested by similar findings in DKO colon cancer cells genetically disrupted for two major DNMTs (*DNMT1*$^{-/-}$, *DNMT3B* $^{-/-}$) versus parental, wild-type HCT116 cells (Figure 1A). The induced responses may not be a general stress phenomenon as they did not occur with carboplatin, a cytotoxic agent commonly used in EOC treatment (Figure 1B). Aza and Dac incorporate into DNA, inhibiting three DNA methyltransferases, but Aza also incorporates into RNA, inhibiting the RNA methyltransferase DNMT2 (Schaefer et al., 2009). Aza thus can demethylate RNA, and unmethylated RNA may activate TLR3 and the interferon response (Karikó et al., 2005). However, Dac and Aza both mimicked the DKO cell line results (Figures 1A, 1C, 1D, S1A, and S1B) strongly suggesting that the drugs directly target DNA methylation to trigger the interferon response.

Aza and Dac similarly triggered an interferon response that includes interferon beta (*IFNβ1*) and a panel of interferon-stimulated genes (ISGs) (*IFI16*, *IFI27*, *IFI44*, *IFI44L*, *MX1*, and *OASL*) (Figures 1C, 1D, S1A, and S1B). Each ISG functions predominantly in anti-viral and anti-proliferative signaling (Figure 2A; Ivashkiv and Donlin, 2014). In four EOC cell lines, key upstream genes in the type I Interferon pathway (*IFNβ1*, *IRF7*, and *STAT1*) were generally upregulated at the fourth day following the end of Aza treatment ("day 7") and further increased by day 10 (Figures 1C, 1D, S1A, and S1B). Importantly, cytosolic sensors for DNA (*MB21D1*/CGAS and *TMEM173*/STING) and RNA (*DDX41*, *DDX58*/RIG-I, and *IFIH1*/MDA5) were also variably upregulated (Figures 1A, 1C, 1D, S1A, and S1B). In A2780 and TykNu, but not Hey or Kuramochi, cell lines, variable increases occurred in type III interferon signaling genes, also involved in response to viruses (Ding and Robek, 2014). These included *IFNL1* (IL28A) and *IFNL3* (IL29) ligands (Figure S1C) and especially the IFN III receptor *IFNLR1* (Figure S1C), known to be methylated and activated by epigenetic therapy (Ding et al., 2014).

Key viral RNA sensing proteins include TLR3 on the endosomal membrane and MDA5, PKR, and RIG-I in the cytoplasm (Figure 2A). These induce IRF3, IRF7, and NF-κB to translocate to the nucleus and activate transcription of *IFNβ1* (Ivashkiv and Donlin, 2014). *IRF7* is frequently promoter DNA hypermethylated in cancer and the associated low basal expression can be reversed by Aza in squamous NSCLC (Wrangle et al., 2013). Among 23 EOC lines examined, *IRF7* was hypermethylated in only one, A2780 (Li et al., 2014) (Figure S3A), potentially not a classic high-grade EOC (Anglesio et al., 2013;

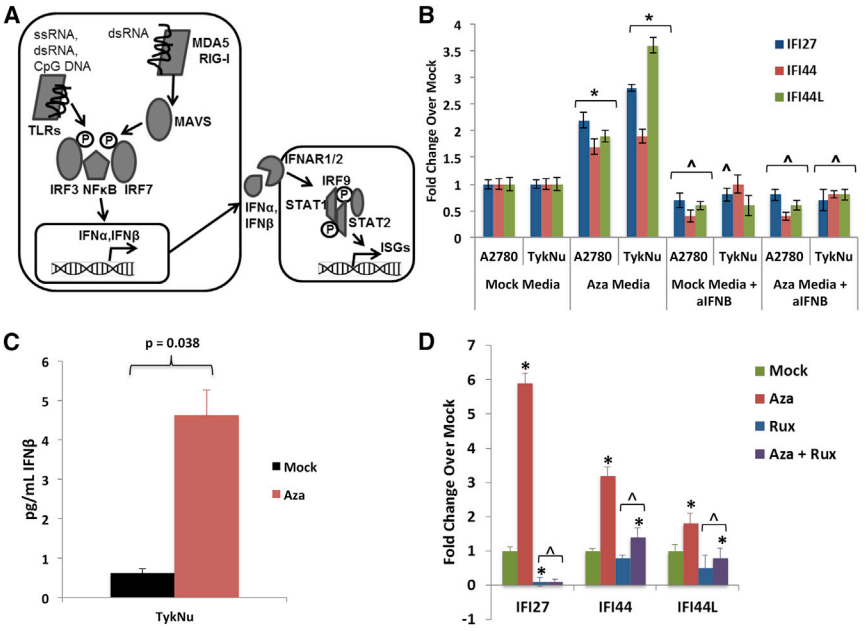

Figure 2. DNMTis Upregulate Immune Signaling through Secreted Interferon

(A) Schematic of interferon pathway. Protein symbols outlined in text.

(B) Treatment of recipient A2780 or TykNu cells with media from cells treated with Mock or Aza, +/− addition of anti-IFNβ. y axis, qRT-PCR fold Aza/Mock of ISGs. *$p \leq 0.05$ and ^$p \leq 0.05$, respectively, for Mock or Aza media with versus without anti-IFNβ versus Mock or Aza media plus anti-IFN β.

(C) ELISA of IFNβ in media from TykNu cells at day 10 from studies in (B). y axis, pg/mL IFNβ.

(D) Treatment of EOC cells with Aza as in Figures 1C and 1D, but in the presence of 2 μM Ruxolitinib (Rux). y axis, qRT-PCR fold changes for ISGs. *$p \leq 0.05$ for fold Aza over Mock and ^$p \leq 0.05$, Mock or Aza versus Mock + Rux or Aza + Rux. Data, mean ± SEM of three (B and D) or four (C) biological replicates.

See also Figure S2.

Domcke et al., 2013). Aza induced partial *IRF7* demethylation and increased expression in this cell line at days 7 and 10 while carboplatin did not (Figures 1B, 1C, and S3A), and *IRF7* knockdown significantly reduced the Aza interferon response (Figures S3B and S3C). Such *IRF7* induction did not occur in two EOC lines or the HCT116 colon cancer cell line where the gene is not hypermethylated (Figures 1D, S1A, S1B, and S3A).

When *IRF7* is not silenced, other mechanisms must then be operative for Aza to trigger viral defense signaling. Secreted IFNβ is critical to this signaling and, through interaction with surface receptors IFNAR1/2, activates JAK/STAT signaling, transcription of ISGs, and resultant translation inhibition and apoptosis (Platanias, 2005; Ivashkiv and Donlin, 2014) (Figure 2A). Indeed, media transferred to untreated cells from Aza-treated cells 7 days after drug withdrawal caused an interferon response with increased expression of ISGs *IFI27*, *IFI44*, and *IFI44L* (Figure 2B). Moreover, Aza treatment induced secreted IFNβ in media (Figures 2C and S2A) and an IFNβ blocking antibody significantly blocked the Aza-induced ISG media response (Figure 2B). Like type I IFN signaling, type III IFN signaling can be activated by viral infection (Robek et al., 2005; Ding and Robek, 2014). However, even though we observed upregulation of type III ligand transcripts *IFNL1* (IL28A) and *IFNL3* (IL29) (Figure S1C), secreted type III interferon proteins were undetectable by ELISA (Figure S2B).

Aza appears to activate type I, IFNβ-mediated signaling through JAK/STAT, as the JAK/STAT inhibitor ruxolitinib strongly reduced ISG responses (Figures 2D and S2C). Further, antibody blocking of IFNAR2, the IFNβ receptor, abrogated Aza induction of *IFI27*, *IFI44L*, and *IFI6* transcription (Figures 3A and S2D), as did inhibition of IFNβ itself (Figures 3B and S2E). In contrast, blocking the type III interferon, IL10RB receptor, gave only a modest block of *IFI27* increase (Figure S2F). IFNβ binding to IFNAR2 also may contribute to late, Aza-induced apoptosis that peaks at 4–7 days after Aza withdrawal, since anti-IFNAR2 led to a lower ratio of cleaved/total PARP (Figures 3A, 3C, and S2D and S2G).

DNMTis Trigger Viral Defense through Induction of dsRNA

Aza-induced viral defense genes and *IFNβ1* are not generally DNA methylated at promoter regions (Li et al., 2014), thus Aza may activate the pathway upstream of these genes. We considered increases in dsRNA, viral single-stranded RNA (ssRNA), and unmethylated CpG DNA that might trigger cytosolic sensors (Sun et al., 2013). Indeed, 3 days after ending Aza treatment of A2780 and TykNu ovarian cancer cells and subsequent transfection into HT29 colon cancer cells, known to have a robust interferon response (Chiappinelli et al., 2012), cytoplasmic total RNA (without rRNA) and PolyA$^+$ RNA, but not PolyA$^-$ RNA or DNA, increased *IFNβ1* transcripts (Figure 3D) and downstream ISGs (Figures 3E and S3D). Further, RNaseIII treatment of the cytosolic nucleic acids, which specifically digests dsRNA, eliminated the *IFNβ1* upregulation (Figure 4A), but this was not seen with RNaseH treatment that digests DNA-RNA hybrids (Figure S4A).

If dsRNA is required for the above Aza effects, then the key cytosolic sensors, TLR3, MDA5 (*IFIH1*), and RIG-I (*DDX58*), the latter two signaling through the mitochondrial protein, MAVS, should be involved in subsequent *IFNβ1* induction (Figure 2A). Aza increased transcript (Figure 1A) and protein levels for these (Figure 4B). However, RIG-I, which requires a 5′ triphosphate group on RNA for activation, is likely not a key player since alkaline phosphatase treatment of cytosolic nucleic acid fractions did not abolish *IFNβ1* upregulation (Figure S4D). In contrast, knockdown of TLR3 and MAVS in A2780 cells (Figure 2A) decreased Aza upregulation of interferon genes *IFNB1*, *IFI44*, *IFI44L*, and *IFI27* by 2-fold as did MAVS knockdown (Figures 4C, 4D, and S4B). In TykNu, knockdown of TLR3 and MAVS significantly blunted Aza induction of these gene responses

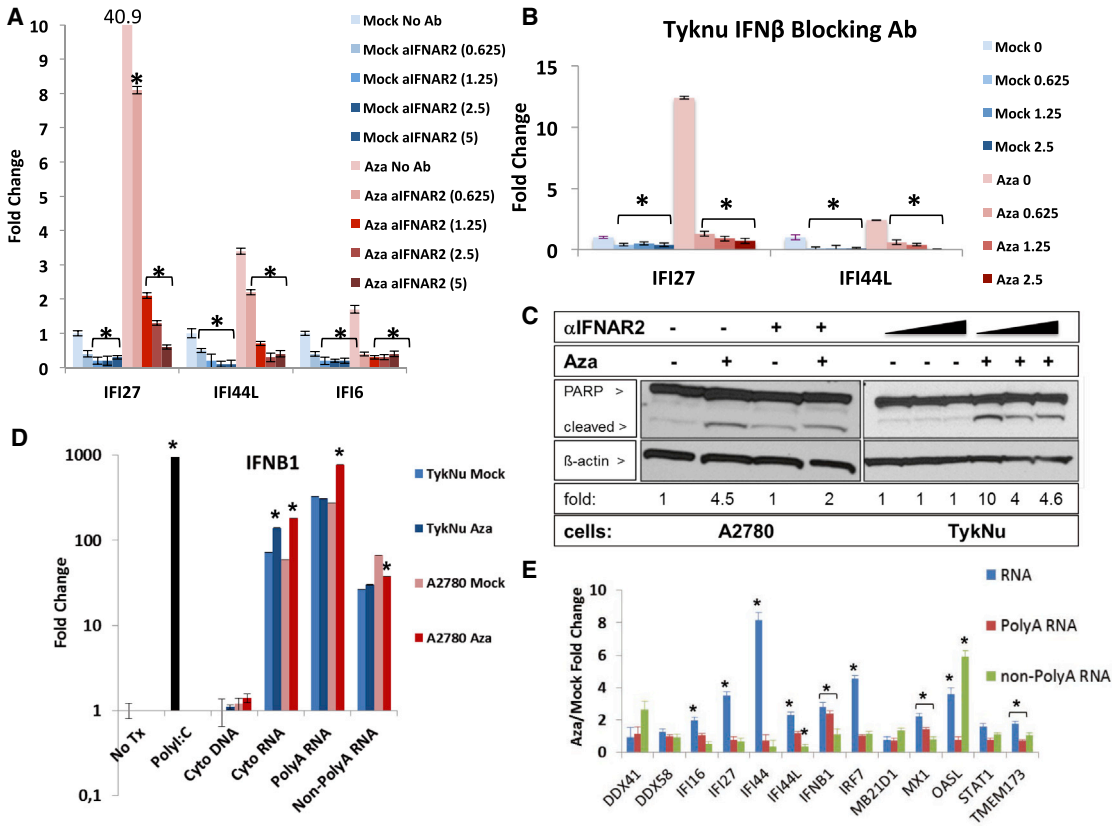

Figure 3. Aza Induces Immune Signaling through dsRNA Activation of Secreted Interferon

(A and B) Blocking (A) IFNAR2 (αIFNAR2) or (B) IFNβ in TykNu cells treated versus non-treated with Aza as in Figures 1C and 1D; parentheses, U/ml of antibody. y axis, qRT-PCR for ISGs. *p ≤ 0.05 for (A), Mock or Aza −anti-IFNAR2 versus +anti-IFNAR2, (B) Mock or Aza with no anti-IFNβ versus with anti-IFNβ.

(C) Immunoblotting for cleaved PARP with β-actin loading control. Fold change shown for cleaved/total PARP ratio, normalized to β-actin for each dose of anti-IFNAR2 (triangles, 0–1.25 U/ml). Aza compared to Mock = 1. The A2780 and TykNu cell lysates were run on two separate gels to perform western blots. The space in between the PARP and B-Actin proteins was cropped out to conserve space.

(D and E) Indicated nucleic acids from the cytoplasm of A2780- or TykNu-treated cells with no drug (Mock) or 500 nM Aza (Aza) for 3 days and rested without drug for 4 days before transfection into recipient HT29 cells. y axis, fold change, Aza/Mock, for *IFNβ1* transcript (D) or ISG transcripts (E) induced in HT29s. No Tx, no transfection; Cyto DNA, cytoplasmic DNA; Cyto RNA, cytoplasmic RNA excluding rRNA. *p ≤ 0.05 for (D) fold change Aza versus Mock, (E) Aza/Mock. Data in (A), (B), (D), and (E) are mean ± SEM of three biological replicates.

See also Figure S3.

(Figure S4C). Importantly, knock down of STING, the cytosolic DNA sensor (Mankan et al., 2014) did not blunt Aza-induced interferon signaling (Figures 4C and 4D). A previous report had implicated STING in viral cytosolic sensing in B cells, but this was dependent upon viral reverse transcriptase activity, likely to be low in our cells (Mankan et al., 2014). We thus conclude that MAVS and TLR3 are centrally involved in Aza triggering cytosolic sensors to induce an interferon response.

Aza-Induced Human Endogenous Retrovirus Transcripts Can Activate Viral Defense Responses in EOC

The above data suggests Aza might activate endogenous retroviral sequences (ERVs) that constitute more than 8% of the human genome, can activate cytosolic RNA sensors, and are silenced in normal somatic cells by promoter DNA methylation (Bannert and Kurth, 2004; Tristem, 2000; Hurst and Magiorkinis, 2014; Mankan et al., 2014). Some cancers lose ERV DNA methylation and aberrantly overexpress ERVs (Larsen et al., 2009; Rycaj et al., 2015; Strick et al., 2007; Strissel et al., 2012; Wang-Johanning et al., 2001, 2007) while others maintain silencing. Aza can induce specific ERV transcripts in melanoma, choriocarcinoma, and endometrial cancer cells (Laska et al., 2013; Ruebner et al., 2013; Stengel et al., 2010; Strissel et al., 2012). Indeed, in initial testing, the *ERVK* subfamily (Wang-Johanning et al., 2003) transcripts increased 2.5-fold in the A2780 cell line upon Aza treatment (data not shown). Upregulation of individual ERVs (22 full-length *env*, 6 partial coding *env*, 1 full-length *gag*, and 2 partial coding *pol*s) (Tables S1 and S2), in PCR assays for non-repeat sequences, occured especially at day 7 (coinciding with ISG expression) in three EOC lines following Aza and Dac treatment (Figures 5A, 5B, and S5A–S5C). These included several known ERV *env* genes like *Syncytin-1*, *ERV-3*, *env-K*, and *env-H* (Blond et al., 1999; Löwer

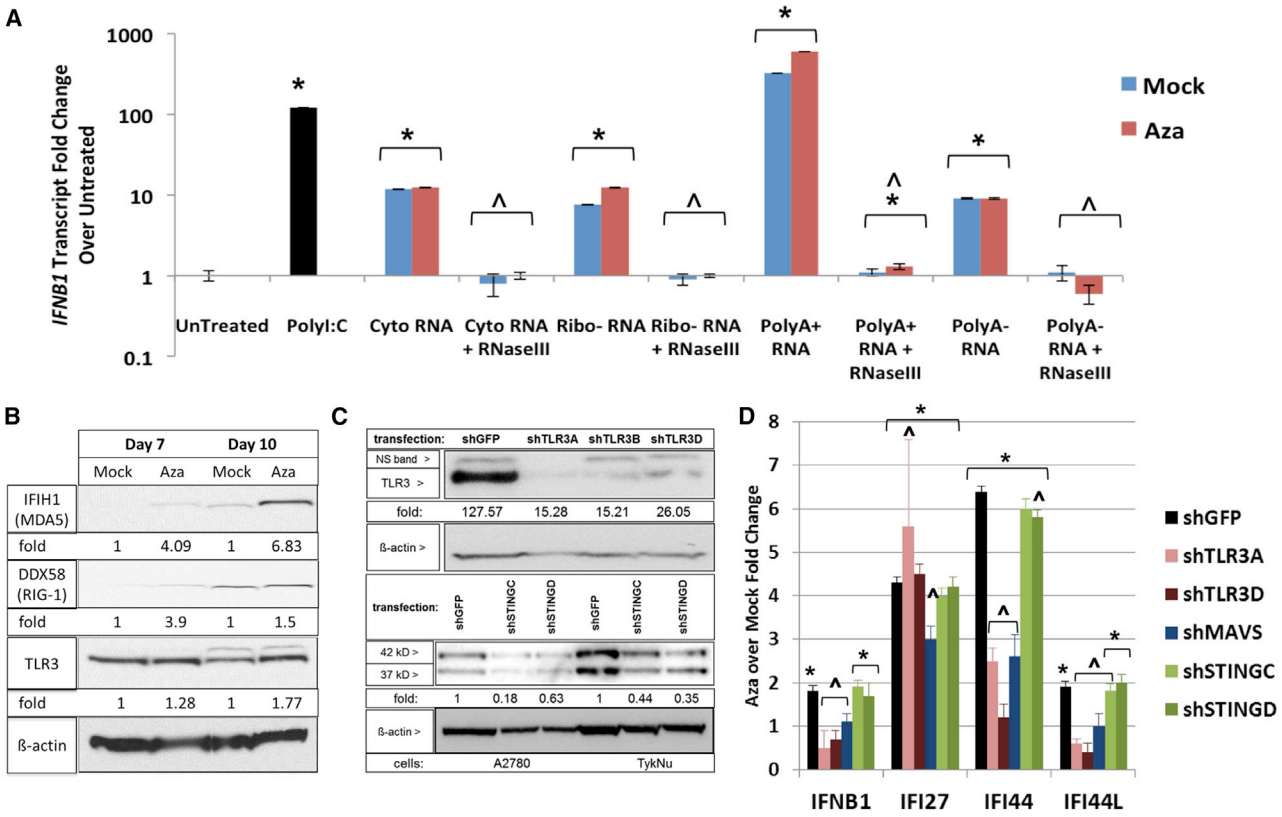

Figure 4. Aza Activates dsRNA Sensors to Induce Interferon Signaling

(A) Effects on *IFNβ1* transcripts, at 24 hr, in HT29 recipient cells transfected with nucleic acid fractions, treated with RNaseIII, from A2780 as in Figures 3D and 3E. *IFNβ1* transcripts were measured at 24 hr. y axis = IFNB1 fold change, *$p \leq 0.05$ for fold change over untreated; ^$p \leq 0.05$ for Mock or Aza + versus − RNaseIII.

(B) Western blots for MDA5, RIG-I, and TLR3 in A2780 cells at four (day 7) and seven (day 10) days after Mock versus 500 nM Aza (Aza) for 3 days. The same A2780 lysate gel was blotted for MDA5 and β-actin, then stripped and reprobed for RIG-I and TLR3. The space between the MDA5, RIG-I, and TLR3 and β-actin proteins was cropped out to conserve space.

(C) Knockdown upon lentiviral infection, with puromycin selection, of A2780 and TykNu with shGFP, shTLR3, and shSTING hairpins. Immunoblotting with anti-TLR3, anti-STING, and anti-β-actin. Densitometry fold change, normalized to mock or shGFP, shown at the bottom of the gels. The shTLR3 and shSTING cell lysates were run on two separate gels to perform western blots. The space in between the TLR3 and β-actin proteins was cropped out to conserve space. The STING western blot was stripped and reprobed with β-actin as a loading control.

(D) qRT-PCR for ISGs from (B) and (C). *$p \leq 0.05$ Aza over Mock; ^$p \leq 0.05$ shGFP versus each shRNA sensor with mean fold change ± SEM of three biological replicates.

See also Figure S4.

et al., 1993; Mi et al., 2000; Rote et al., 2004) and at especially high levels, *env-Fc2*, a less well-characterized gene (Bénit et al., 2003). Finally, in DKO as well as Aza-treated A2780 and TykNu cells, loss of *env-Fc2* promoter methylation correlated with increased *Fc2* expression (Figures 5B, 6A–6C, and S6A) but not in Hey cells (Figure S6A).

Further linking ERVs with a dsRNA-triggered IFN response, bidirectional transcription producing sense and anti-sense transcripts occurred for *Syncytin-1* and five *env-Fc2* gene loci, but not β-actin, in three EOC lines and HCT 116 and DKO cells (Figure 5C; Table S2), analyzed by the TAG-aided sense/antisense transcript detection (TASA-TD) technique (Henke et al., 2015). Such sense and antisense transcripts can form dsRNA (Faghihi et al., 2008; Su et al., 2012). Interestingly for TykNu, there was a 6.69-fold increase of *env-Fc2* antisense transcript levels compared to the sense transcript (Figure 5C) but substantially lower antisense transcripts were seen in both HCT116 and DKO cells (Figures 5C and 6A). Disrupting DNMTs seems integral to the above ERV upregulation since increases of *env-Fc2* and *erv9-1* occurred in DKO versus wild-type HCT116 cells (Figure 6A).

ERV transcripts seem directly involved in the Aza responses that, first, although drug-induced upregulation of ERV transcripts began early after Aza, both ERVs and viral defense gene increases generally peaked by day 7 (Figures S6D and S6J). Second, ERV env proteins such as Syn1 and ERV-3 were not increased after Aza treatment, supporting a dominant role for viral defense signaling via RNA transcripts (Figures 6D, S6B, and S6C). Third, overexpression of *ERV-3*, *EnvW2*, and *Syncytin-1* in TykNu (Figures 6E and 6J), A2780 (Figure S6D), and Hey cells (Figures S6E and S6J), as compared to control genes, increased the same interferon genes induced by Aza (*IFNβ1*,

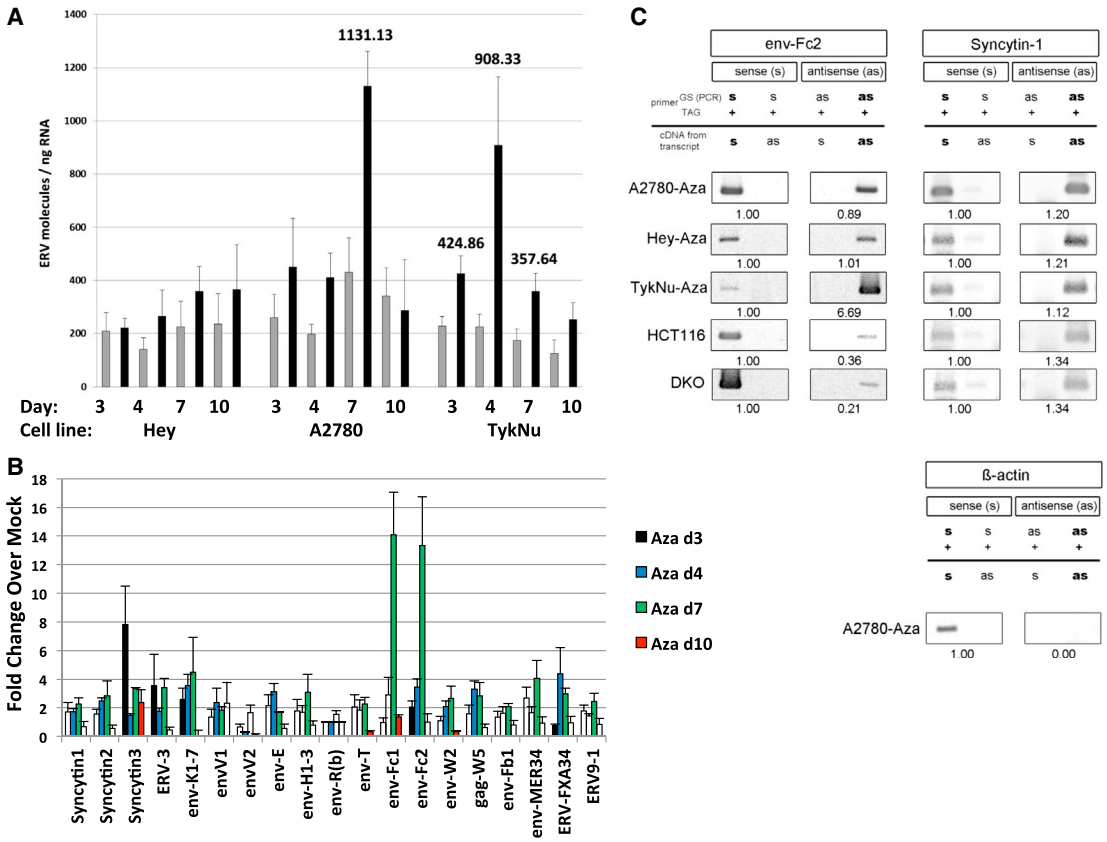

Figure 5. Aza Upregulates Sense and Antisense ERV Transcripts

RNA was isolated from cells at last (day 3), one (day 4), three (day 7), and seven (day 10) days after Mock or 500 nM Aza (Aza) for 3 days.

(A) Total number of molecules for all ERV genes (y axis = ERV molecules/ng RNA). Error bars, SEM for four independent experiments; numbers above bars, significant data for indicated days; gray, Mock; black, Aza.

(B) qRT-PCR of ERV genes in A2780 cells for four independent experiments. y axis, fold increases for Aza/Mock ± SEM and normalized to Mock = 1. White bars, non-significant; colored bars, significant ERV gene induction ($p < 0.05$).

(C) TASA-TD PCR amplified sense and antisense transcripts of the *env-Fc2* (731 bp) and *Syncytin-1* (202 bp) genes from first strand cDNA. Aza-treated A2780, Hey, and TykNu, and HCT116 and DKO cells are indicated. Ratios of sense (s) and antisense (as) determined by ImageJ. PCR primers, gene-specific (GS); TAG. β-actin sense 399 bp amplification product = negative control for as transcripts (Chen et al., 2004). The products from TASA-TD PCR were run on the same gel, then cropped and presented.

See also Figure S5.

IFI27, and *IFI44L*). The increases often exceeded that for the drug likely because total ERV RNA molecules were higher in the overexpression experiments (Figures S6F–S6I). Finally, although siRNA knockdown of individual ERVs (*Syncytin-1*, *ERV-3*) during Aza treatment produced more complex results, targeting two ERVs significantly blunted the Aza-induced gene expression of *IFI27*, *IFI44L*, and *IFI6* in TykNu cells, but not A2780 or Hey cells (Figure S7A).

Importantly, a driving role for ERV transcripts in triggering Aza-induced viral defense gene responses is evidenced by a high correlation of basal levels of both in 19 primary EOC. Total molecules of 22 ERV *env* genes queried were increased ($p < 0.05$) in tumor versus normal ($n = 9$) and divided tumors into lower ($n = 9$) and higher ERV ($n = 10$) expression groups as compared to normal controls. High ERV tumors had significantly higher viral defense response gene expression ($p = 0.000141$) (Figure 7A).

Viral Defense Gene Levels Divide Human Tumors into High and Low Expression Groups that Track with Responses to Immune Checkpoint Therapy

Human cancers can evolve immune evasion to become less responsive to immune modulation (Drake et al., 2006; Schreiber et al., 2011). In this regard, basal transcript levels for the Aza-induced viral defense genes grouped primary EOC, breast, colon, and lung cancers, and melanoma from The Cancer Genome Atlas (TCGA) studies into high and low groups (Figures 7B and S7C–S7F). For EOC, this basal expression divided tumors into high, medium, and low expression groups and the former two encompass virtually all of the TCGA (Verhaak et al., 2013) immune reactive (IMR) good prognosis tumors. The Low group encompasses the PRO (high proliferative), poor prognosis subtype ($p < 0.001$ to 0.0001) (Figures 7B and S7B). Interestingly, virtually all of the right-sided colon cancers with a high DNA hypermethylation frequency phenotype ($p < 0.002$), termed CIMP, which have a high

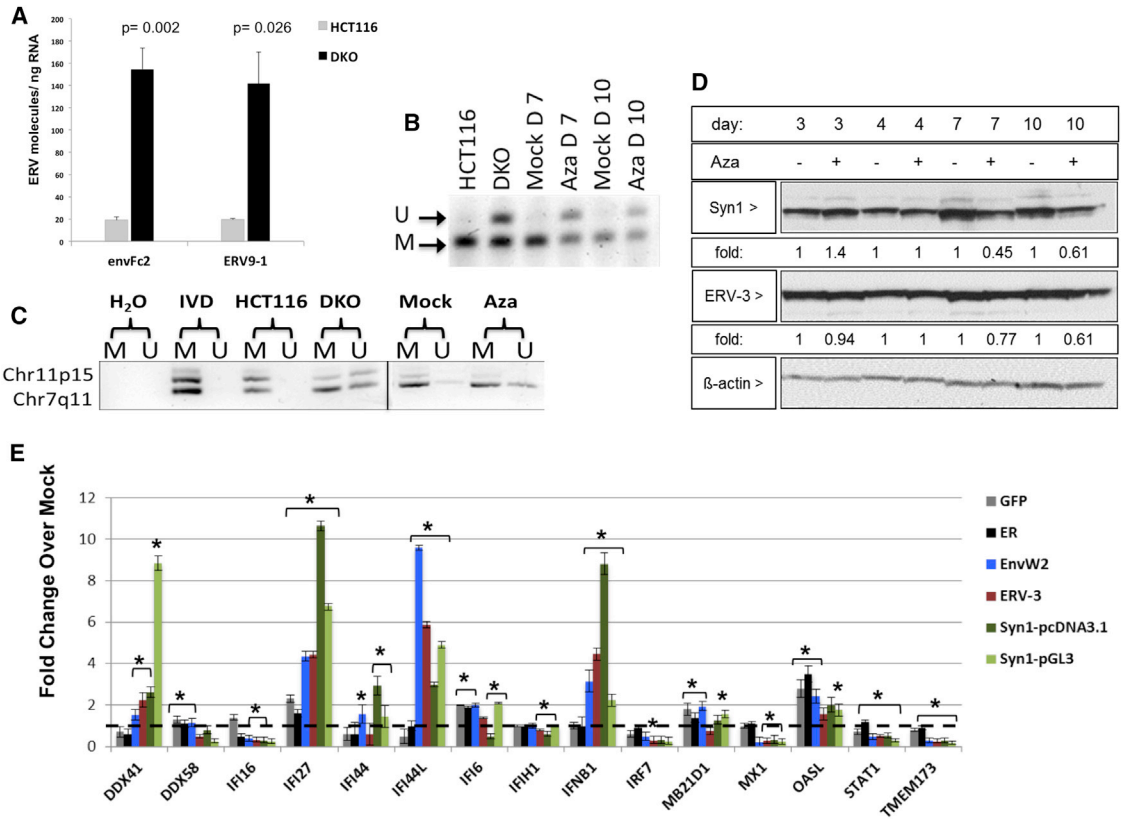

Figure 6. Aza Upregulates ERV Transcripts, but Not Proteins, through DNA Demethylation

(A) *env-Fc2* and *erv-9-1* ERV gene total number of molecules, assayed by qRT-PCR, for DKO (DNMT1$^{-/-}$, DNMT3B$^{-/-}$) and parental HCT116 cells. y axis, mean ± SEM for n = 6 biological replicates.

(B) DNA methylation changes in ERVs in A2780 cells treated with Mock or 500 nM Aza for 3 days at post-treatment day 4 (day 7) or 7 (day 10). Bisulfite-treated DNA was amplified and digested with the AciI enzyme producing 155 and 44 bp fragments of methylated DNA while unmethylated DNA does not digest (199 bp fragment). U, unmethylated band; M, methylated band.

(C) DNA from (B) was subjected to methylation-specific PCR for ERV-Fc2 family members on chromosomes 7 and 11. U, unmethylated; M, methylated; IVD, in vitro methylated DNA. The products from the same MSP reaction were run on two different gels and presented together. Vertical line indicates distinction between gels on the left and right.

(D) Syncytin-1 and ERV-3 protein levels in EOC cells treated as in (B). Fold change for densitometry by ImageJ for Aza versus Mock cells normalized to β-actin protein levels. The same gel blotted for Syncytin-1 and β-actin, then stripped and reprobed for ERV3. The space between the Syncytin-1 and ERV3 and the β-actin proteins was cropped out to conserve space.

(E) Transfection of full-length *env* genes from EnvW2, ERV-3, or Syncytin-1 or EGFP and ER controls in TykNu cells. qRT-PCR was performed for ISGs 7 days after transfection. Dotted black line indicates 1. Y axis, mean ± SEM fold change of three biological replicates for overexpression/Mock. *p ≤ 0.05.

See also Figure S6.

burden of DNA mutations and respond robustly to immune checkpoint therapy (Le et al., 2015), were in the High and Intermediate groups (Figure S7C). High mutation burden has recently been defined as a key correlate to response to immune checkpoint therapy (Rizvi et al., 2015; Snyder et al., 2014). Similar sharp high versus low clustering is seen for subgroups of breast and lung cancers and melanoma (Guan et al., 2015) (Figures S7D–S7F), the last being very responsive to immune checkpoint therapy (Topalian et al., 2015; Hodi et al., 2010; Weber et al., 2015).

Could the levels of viral defense pathway signaling correlate with improved responses to immune checkpoint therapy? Indeed, for RNA-seq transcriptomes of melanoma patients treated with anti-CTLA-4, high levels of the viral defense signature expression in tumor samples correlated with long term benefit (disease control [stable disease or better] >6 months as measured radiographically) in patients treated with anti-CTLA-4 therapy (Snyder et al., 2014) (Figures 7C and 7D; Tables S5 and S6). Importantly, high viral defense signature again correlated with high mutational burden (Figure 7C).

Aza Treatment Potentiates Immune Checkpoint Therapy in a Mouse Model of Melanoma

In the B16-F10 mouse melanoma model, multiple combinations of low dose Aza directly enhanced tumor responses to anti-CTLA4 immune checkpoint therapy (Figures 7E, 7F, and S7G). Further, B16 cells treated in vitro with Aza, then injected into mice and treated with anti-CTLA-4, were cleared completely (data not shown). Thus DNMTis can potentiate the anti-tumor effects of immune checkpoint inhibitors.

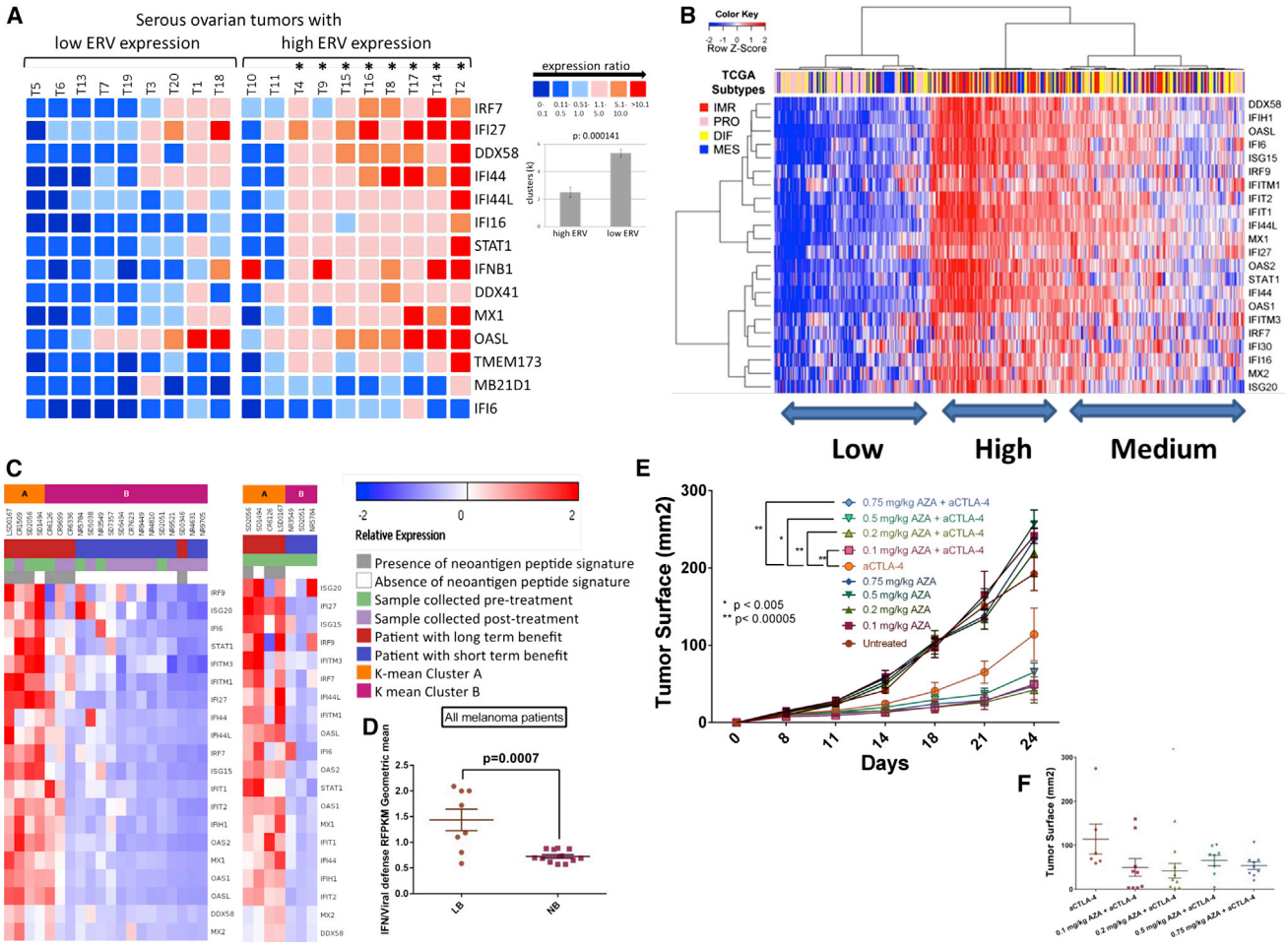

Figure 7. Aza-Upregulated Viral Defense Genes Are Significantly Correlated with ERVs in Primary Tumors and Correlate with Sensitivity to Immune Therapy

(A) Heatmap comparing basal levels of viral defense genes and ERVs in primary EOC. The cut-off for lower or higher ERVs was the mean control tissue value of 237.57 ± 83.05 molecules/ng RNA. Mean ISGs of the high ERV ovarian tumor (T) cohort (n = 10) is 12.65-fold higher than the mean of ISGs of the low ERV cohort (n = 9). The (*) denotes that eight of ten high ERV tumors had significantly higher ISG expression compared to the low ERV tumors. ISG expression is organized according to low and high ERV expression cohorts in arbitrary units; color code from blue to red shows increasing ISG expression. For clusters (k = 6), differences are significant between the high ERV expression (2.5 ± 0.37) and the low ERV expression cohort (5.33 ± 0.28).

(B) Interferon-stimulated viral defense genes upregulated at least 2-fold by Aza in EOC cell lines (right y axis) were used to cluster EOC tumors for RNA-seq data (blue, low; red, high) from The Cancer Genome Atlas (TCGA). EOC TCGA subtypes are shown: DIF (differentiated), IMR (immune reactive), MES (mesenchymal), and PRO (proliferative).

(C and D) Viral defense gene signature is upregulated in tumors from anti-CTLA-4-treated metastatic melanoma patients who derived durable clinical benefit (complete response, partial response, or progression free-survival >6 months as previously described) (Snyder et al., 2014) compared to those without benefit. Tumors collected pre-CTLA-4 treatment and shortly post-treatment are shown.

(D) y axis = RFPKM mean of viral defense genes in all melanoma patients.

(E and F) Tumor responses of mice injected with B16-F10 cells and treated with either PBS, anti-CTLA-4, Aza, or both anti-CTLA-4 and Aza. Data represent results from one of two independent experiments with identical results, each with n = 10 per arm. Y axis = mean tumor surface, error bars ± SEM.

See also Figure S7 and Tables S5 and S6.

DISCUSSION

Our present data now provide functional context for our earlier reports that DNMTis induce a complex set of immune pathway responses in tumor cells (Li et al., 2014; Wrangle et al., 2013). DNMTis trigger cytoplasmic dsRNA sensing, central to cellular viral defense responses, and activate interferon in EOC and colon cancer cells by disrupting DNMTs. This activation could induce tumor attraction of lymphocytes (Ivashkiv and Donlin, 2014). There are some important implications for one of the most exciting new developments in cancer treatment, immune checkpoint therapy (Brahmer et al., 2010, 2012; Berger et al., 2008; Leach et al., 1996; Topalian et al., 2015; Hodi et al., 2010; Weber et al., 2015) and for underlying mechanisms inherent to both tumor and host cells for reversal of immune tolerance in tumor infiltrating T-lymphocytes (Pardoll, 2012).

Indeed, basal levels of our viral defense gene transcripts divide EOC, and other major cancer types in TCGA, into low and high expression subgroups. Perhaps most intriguingly, such high basal expression in tumors tracks with favorable patient responses in a trial of immune checkpoint therapy for advanced melanoma, and strong Aza sensitization to immune checkpoint therapy is seen in a pre-clinical mouse melanoma model.

A major trigger of the Aza-induced viral defense response appears to be bidirectional transcription of ERVs that are known to fold into dsRNA secondary structures. ERVs, representing more than 8% of the human genome (Bannert and Kurth, 2004; Tristem, 2000), integrated into the genome of mammals between 0.1 and 40 million years ago via exogenous retroviral infections of germ cells (Egan et al., 2004; Turner et al., 2001). Most ERV genes are non-functional due to DNA recombination, mutations, and deletions, but some produce functional proteins including group-specific antigen (gag), polymerase (pol) with reverse transcriptase (RT), and the envelope (env) surface unit (SU) with a transmembrane immunosuppressive-like peptide (Mi et al., 2000; Blaise et al., 2005; de Parseval et al., 2003; Villesen et al., 2004). The *env* gene of *ERVW-1* (chromosome 7q21.2) called *Syncytin-1* has an essential role in placentogenesis (Blond et al., 1999; Mi et al., 2000).

Importantly, and key to our findings, a major function of DNA methylation in humans is silencing of ERVs and other viral sequences in the human genome; up to 90% of methylated CpGs are located in 45% of the human genome harboring repetitive elements like ERVs (Walsh et al., 1998; Bestor and Tycko, 1996). However, ERV genes are unmethylated and expressed in embryonic stem cells (Santoni et al., 2012) and especially *Syncytin-1* is epigenetically regulated throughout placentogenesis (Matousková et al., 2006). Some tumors have ERV demethylation and increased expression such as the ERV-K (HML-2) 5′LTR-UTR in melanoma (Stengel et al., 2010) and the 5′-LTR region of several ERVs in testicular cancer (Gimenez et al., 2010). A 20% overall mean demethylation of single CpGs in the ERVW-1 5′ LTR regulating *Syncytin-1* correlates with increased expression in endometrial cancer (Strissel et al., 2012). Indeed, ERVs can be targeted as tumor-associated antigens on melanoma cells (Cooper et al., 2015). In contrast, as in our present data and those of others (Maksakova et al., 2008), in some cancers, individual ERVs can maintain full or partial promoter DNA methylation and low expression and DNMTis can induce ERV demethylation and viral defense signaling in human embryonic stem cells (Grow et al., 2015).

In addition to ERVs, other noncoding RNAs could contribute to the Aza-induced immune response, such as repetitive Alu elements (Tarallo et al., 2012). UV light can damage small nucleolar RNA and activate an interferon response via TLR3 (Bernard et al., 2012) and very high dose (10 μM) Dac can induce an interferon response, apoptosis, increased ERVs and repetitive satellite RNAs in p53 null mouse fibroblasts (Leonova et al., 2013). We suspect, however, that such high Dac doses induce DNA damage rather than simply epigenetic effects. Half of the ovarian cancer lines we studied (Li et al., 2014) have wild-type *P53* but we see no differences in Aza interferon response between these and those with mutant *P53*.

The high translational connotations of our findings, including the small number of patients in clinical trials for NSCLC who may have been sensitized by epigenetic priming to immune therapy (Wrangle et al., 2013), remain to be validated in larger clinical trials. These are ongoing for NSCLC (Brahmer, 2015) and planned for advanced ovarian cancer. Moreover, ERV-K env proteins have been shown to increase immunotherapeutic potential of melanoma, breast, and ovarian cancer patients (Rycaj et al., 2015; Wang-Johanning et al., 2012; Cooper et al., 2015). Also, our hypotheses that drugs like Aza might sensitize patients with multiple cancer types to immune checkpoint blockade and other immunotherapies are further strengthened by the data in our pre-clinical melanoma model. For immune checkpoint therapy, in addition to the functional significance of our data, a potential biomarker strategy is suggested by our findings in a melanoma trial. The high correlation of viral defense signaling with mutational burden suggests that genetic changes, increases in ERVs, and viral defense genes could predict response to immune checkpoint and other immunomodulatory approaches. Finally, our drug approach to upregulate viral defense signaling might be compared to the use of oncolytic viruses to induce inflammatory immune infiltrates at tumor sites to sensitize to immunomodulation (Zamarin et al., 2014).

EXPERIMENTAL PROCEDURES

Detailed materials and methods can be found in the Supplemental Experimental Procedures.

Cell Line Treatments

Cell lines were treated with 500 nM Aza, 100 nM Dac, or 500 nM-3 μM carboplatin (Sigma) for 72 hr, and DNA and RNA were isolated using standard methods at 1, 3, or 7 days following removal of drug. Ruxolitinib (2 μM) (Invivogen tlrl-rux), 0.625–5 U/ml of anti-IFNAR2 antibody (PBL Interferon Source 21385-1), 0.625–2.5 U/ml of anti-IFNB antibody (PBL Interferon Source 31400-1), or 1.25–5 U/ml of anti-IL10RB antibody (Abcam ab89884) were added during DNMTi treatment. Preparation of nuclear and cytoplasmic fractions of cultured cells was performed as described (O'Hagan et al., 2011). Ribosomal RNA was depleted using the Ribominus kit (Invitrogen), and PolyA$^+$ and PolyA$^-$ RNA were isolated using the Oligotex Direct mRNA Mini Kit (Invitrogen). Nucleic acids were treated with 1 U/μg of RNase III (Ambion), 10 U/μg of RNaseH (Invitrogen), or 3 U/1 μg calf intestine alkaline phosphatase (New England Biolabs) according to manufacturer's instructions, and 400 ng of each nucleic acid was transfected into HT29 cells.

DNA Methylation Analysis

DNA was bisulfite converted and subjected to methylation-specific PCR (Herman et al., 1996) for *IRF7* and *ERV-Fc2* and COBRA (Xiong and Laird, 1997) for the *ERV-Fc2* locus on chromosome 11.

Transcript Abundance

Real-time RT-PCR was performed with an Applied Biosystems 7500 Fast Real-Time PCR machine by the $2^{-\Delta\Delta CT}$ method and TASA-TD strand-specific PCR by the method of Henke et al. (2015).

Protein Analysis

Western blot analyses employed antibodies against ERV-3 (1:1,000, Everest), B-actin (1:5,000, Sigma), MDA5 (1:1,000, Cell Signaling 5321), PARP (9542, 1:1,000; Cell Signaling Technology), RIG-I (1:1,000, Cell Signaling 4200), STING (1:1,000, Abcam ab82960), Syncytin-1 (1:350, Dr. Hervé Perron, Geneuro, Geneva Switzerland), and TLR3 (1:1,000, Cell Signaling 6961). IFNB

ELISA utilized the *Verikine-HS* Human Interferon Beta Serum ELISA kit (PBL Interferon Source) and IFNL ELISA the DuoSet ELISA for Human IL-29/IL28-B (IFNL 1/3) kit (R&D Systems).

Knockdown and Overexpression Experiments

Syncytin-1, *ERV-3* and *ERV-W2 env*, *Estrogen receptor alpha (ER)*, and E-GFP vectors and siRNAs targeting Syncytin-1, ERV-3, or a scrambled control, were transfected using the JetPei or Hyperfect transfection reagents, respectively. TLR3, MAVS, and STING shRNA were performed according to established methods (Stewart et al., 2003).

RNA-Seq Expression Analysis of Tumors from Anti-CTLA-4-Treated Patients

Patients were described previously (Snyder et al., 2014) and samples were obtained with written informed consent per approved institutional review board (IRB) protocols. Expression data were obtained using RNA-seq with all data deposited at The cBio portal under the study name Metastatic Melanoma (MSKCC Cell, 2015).

B16-F10 Melanoma Mouse Model

C57BL/6J mice were subcutaneously injected with 1×10^5 B16-F10 tumor cells. On days 4, 8, 11, 14, and 18, mice were treated intraperitoneally with 100 μg anti-ctla-4. Mice received two cycles of intraperitoneal injection of 0.1 to 0.75 mg/kg Aza in PBS for 5 consecutive days followed by 7 days off treatment, starting at day 8 after developing palpable tumors, with control groups receiving corresponding doses of non-specific isotype antibody control and PBS intraperitoneally. Tumor surface was measured with a caliper using the ellipse surface formula (length × width × π)/400. All mouse procedures were performed in accordance with the institutional protocol guidelines from the Memorial Sloan Kettering Cancer Center (New York, NY).

Statistical Analysis

Mean ± SEM qRT-PCR results were considered statistically significant with p values ≤ 0.05 by Mann-Whitney U test or Student's t tests and two-tailed p values are reported. Tumor growth was assessed by two-way ANOVA between each of the mouse treatment groups with p values adjusted by the Dunnett multiple comparison test (df = 512).

Normalized, level 3 Agilent expression data were obtained from The Cancer Genome Atlas data portal (https://tcga-data.nci.nih.gov/tcga/) and analyzed by R statistical software (http://www.r-project.org) with existing packages and customized routines. Consensus hierarchical clustering was performed with the ConsensusClusterPlus R-package (Wilkerson and Hayes, 2010) and data analyzed by the Fisher exact p value test for association between clusters.

ACCESSION NUMBERS

The expression data reported in this paper is deposited in the cBio portal under the study name Metastatic Melanoma (MSKCC Cell, 2015).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and six tables and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2015.07.011.

AUTHOR CONTRIBUTIONS

K.B.C., P.L.S., A.D., T.M., J.W., T.A.C., S.B.B., and R.S. designed experiments, performed data analyses, and wrote the manuscript. K.B.C., P.L.S., C.H., A.D., A.H., B.A., and S.B. performed experiments. N.S.R. provided ERV-3 *env* cDNA plasmid. D.S. provided ovarian cancer cell lines. H.L., A.S., V.M., D.M.P., L.M.C., M.W.B., and C.A.Z. assisted with data analyses.

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